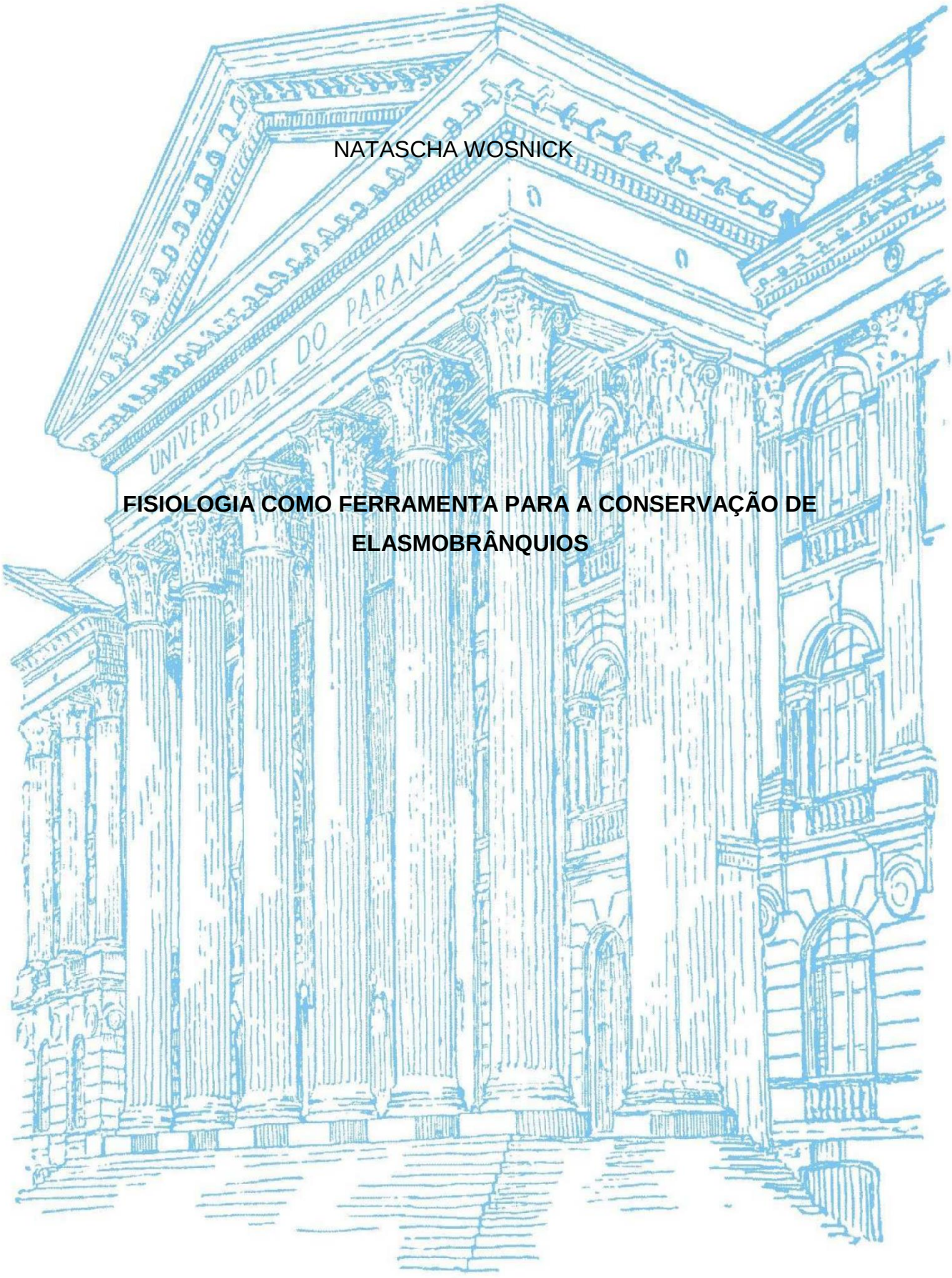
UNIVERSIDADE FEDERAL DO PARANÁ




NATASCHA WOSNICK

# FISIOLOGIA COMO FERRAMENTA PARA A CONSERVAÇÃO DE ELASMOBRÂNQUIOS

CURITIBA

2017

UNIVERSIDADE FEDERAL DO PARANÁ

NATASCHA WOSNICK

# FISIOLOGIA COMO FERRAMENTA PARA A CONSERVAÇÃO DE ELASMOBRÂNQUIOS

Tese de doutorado apresentada como requisito parcial à obtenção do grau de Doutora em Zoologia, no Programa de Pós-Graduação em Zoologia, Setor de Ciências Biológicas, da Universidade Federal do Paraná.

Orientadora: Profª. Drª Carolina Arruda de Oliveira Freire

CURITIBA

2017

Universidade Federal do Paraná
Sistema de Bibliotecas

Wosnick, Natascha
Fisiologia como ferramenta para a conservação de elasmobrânquios. / Natascha Wosnick. – Curitiba, 2017.
152 f., il. ; 30cm.

Orientador: Carolina Arruda de Oliveira Freire

Tese (doutorado) - Universidade Federal do Paraná, Setor de Ciências Biológicas. Programa de Pós-Graduação em Zoologia

1. Raia (Peixe) 2. Tubarão 3. Salinidade 4. Temperatura 5. Pesca 6. Peixe - Fisiologia I. Título II. Freire, Carolina Arruda de Oliveira III. Universidade Federal do Paraná. Setor de Ciências Biológicas. Programa de Pós-Graduação em Zoologia

CDD (20. ed.) 597

MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
Setor CIÊNCIAS BIOLÓGICAS
Programa de Pós-Graduação ZOOLOGIA

# TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ZOOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **NATASCHA WOSNICK** intitulada: **Fisiologia como ferramenta para a conservação de elasmobrânquios**, após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua aprovação no rito de defesa.

A outorga do título de doutor está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

Curitiba, 22 de Junho de 2017.

CAROLINA ARRUDA DE OLIVEIRA FREIRE
Presidente da Banca Examinadora (UFPR)

LUIS FERNANDO FÁVARO
Avaliador Interno (UFPR)

EMYGDIO LEITE DE ARAÚJO MONTEIRO FILHO
Avaliador Interno (UFPR)

RENATO HAJENIUS ACHÉ DE FREITAS
Avaliador Externo (UFSC)

GETULIO RINCON FILHO
Avaliador Externo (UFMA)

## AGRADECIMENTOS

Durante estes quatro anos, foram tantas as experiências e pessoas que cruzaram meu caminho que estes agradecimentos certamente não serão suficientes para expressar minha gratidão a todos aqueles que participaram da minha jornada.

Primeiramente, agradeço à minha orientadora e mentora de vida, Drª. Carolina Freire, pelos nove anos de aprendizado. Obrigada por acreditar em mim, nunca desistir das minhas ideias malucas e por me permitir trilhar meu caminho profisional mesmo este tão distante do foco de pesquisa do laboratório. Palavras não são suficientes para expressar minha gratidão por todo o apoio e ensinamentos.

Agradeço imensamente ao Dr. Neil Hammerschlag, meu orientador durante o período de Sanduíche por todo o apoio e dedicação a mim e ao meu projeto. Fazer parte de sua equipe e ter tido a honra de ser sua aprendiz foram as melhores experiências da minha vida, é impossível expressar minha gratidão e admiração por você.

Agradeço à CAPES pela bolsa de Doutorado concedida e também pela incrível e única oportunidade de desenvolver parte da minha pesquisa no exterior durante meu período de Sanduíche. Agradeço tambem ao Programa de Pós-Graduação em Zoologia e à Universidade Federal do Paraná pela vaga concedida. Por fim, agradeço à Vanessa Peres, Secretária do Programa de Pós-Graduação em Zoologia por toda a ajuda e apoio durante esta jornada.

Ao Dr. Emygdio Leite de Araújo Monteiro-Filho pelo apoio incondicional, sem sua ajuda meu projeto do Sanduíche jamais seria possível. Ao Dr. Carlos Aturo Navas e Dra. Renata Guimarães Moreira, por embarcarem nesse desafio de trabalhar com elasmobrânquios conosco, permitindo que nosso campo de atuação se expandisse enormemente.

Ao Dr. Fabiano Bendhack que desde a minha graduação vem sendo uma base sólida e essencial de apoio para que meus experimentos pudessem ser realizados, seu apoio logistico foi essencial para o desenvolvimento desta tese.

Ao Dr. Fábio Hazin pela incrível e inesquecivel oportunidade de trabalhar com tubarões vivos pela primeira vez em minha vida. Espero poder continuar nossa parceria por muitos anos, você é uma inspiração.

Aos Drs. Getulio Rincon, Luís Fernando Fávaro e Renato Hajenius Aché de Freitas pela participação como membros da banca de defesa e por todas as sugestões que tornaram este trabalho ainda melhor. Admiro demais todos vocês.

Aos meus familiares, em especial minha mãe, mulher maravilhosa e guerreira que sempre me apoiou e incentivou. Ao meu namorado Mateus pelo apoio e companheirismo nas horas dificeis desta fase final.

Aos colegas de laboratório, Silvia Gutierre, Anieli Maraschi, Juliane Ceron, Marcos Paulo, Flávia Sampaio, Eloísa Giaretta, Leonardo Rios, Felipe Brandalize, Ísis Cury, Deivyson Bozza, Gustavo Yamassaki, Guilherme Torres, Daliana Bordin e Luciana Bastos por todo o companheirismo. Agradeço especialmente minha colega e grande amiga Giovanna Castellano por literalmente salvar minha pele em tantos momentos durante o Doutorado.

Por fim, a todos os meus colegas de profissão e grandes amigos Andrey Castro, Hugo Bornatowski, Ana Rita Onodera, Otto Gadig, Vicente Faria, Renata D. Leite, Pedro Calixto, Gabriel Vianna, Aline Poscai, Alexandre Rodrigues, Jones Santader, Rodrigo Barreto, Elizabeth Cavalcanti, Carlolina Ferraz, Natália Alves, André Afonso, Isabella Simões, Maíra Afonso, Dráusio Veras, Fábio Hoppe, Tiego Costa, Julia Whidden, Shannon Moorhead, Jake Jerome, Stephen Cain, Rob Roemer, Nick Perni, Frank Gibson e Pat Goebel. Agradecimento especial à Bianca Rangel, minha maior parceira e colaboradora, sem você minha vida pessoal e professional não seriam as mesmas, palavras não são suficientes para expressar meu agradecimento.

## RESUMO

Predadores de topo e mesopredadores são de extrema importancia para a saúde e equilíbrio dos ecossistemas onde estão inseridos. Desequilíbrios ambientais que causam disrupção ecológica afetam primeiramente animais que ocupam níveis tróficos mais elevedos, sendo estes importântes indicadores (sentinelas) de impacto. Raias e tubarões são constantemente expostos à pressão antropogênica, sendo observada, atualmente, uma redução expressiva das populações em nível mundial. Dentre os impactos antropogênicos com efeitos diretos em elasmobrânquios, a sobrepesca é a atividade mais expressiva, responsável por declínios de até 90% para algumas espécies de grande importância ecológica (e.g. tubarões-branco; tubarões-martelo). Nas últimas décadas, o declínio acentuado de várias espécies de predadores de topo já apresentou efeitos tróficos negativos, através da redução da resiliência de espécies-chave. Adicionalmente, os efeitos das mudanças climáticas na distribuição e resiliência de raias e tubarões já são observados, afetando não apenas o equilíbrio dos ecossistemas, mas também a economia baseada na captura comercial. O uso de ferramentas fisiológicas como método para avaliar a plasticidade e vulnerabilidade é um campo promissor, uma vez que permite uma melhor compreensão da habilidade espécie-específica frente a estressores antrópicos e alterações ambientais. Dessa forma, a fisiologia da conservação pode ajudar a resolver problemas de gestão através da elucidação de processos biológicos básicos moldados evolutivamente, determinando assim como um organismo irá responder à ameaças imediatas. Baseada nesta premisa, esta tese de doutorado teve como objetivo utilizar análises fisiológicas para a análise de mecanismos ativados frente à condições estressoras as quais estes animais são comumente expostos (estresse de captura e manejo; exposição ao ar) ou poderão ser expostos frente à alterações climáticas (alterações térmicas e salinas). Os quarto capítulos iniciais têm como foco de estudo os efeitos deletérios da pesca (comercial e esportiva) em *Zapteryx brevirostris* (capítulo 1), *Galeocerdo cuvier* (capítulo 2), *Ginglymostoma cirratum* (capítulo 3) e *Carcharhinus limbatus* (capítulo 4) com a utlização de ferramentas fisiológicas (análises plasmáticas e varredura infravermelha) de forma a avaliar os mecanismos fisiológicos ativados frente ao estresse de captura, exposição ao ar e manejo. Os dois capítulos finais da tese objetivam a exploração da plasticidade de *Zapteryx brevirostris* (capítulo 5) e *Sphyrna mokarran* (capítulo 6) frente à alterações ambientais previstas pelas mudanças climáticas. Análises plasmáticas e varredura infravermelha também foram utilizadas. Os resultados demonstram que todos os estressores analisados são significativos, alterando os padrões homeostáticos das espécies aqui estudadas, podendo reduzir de forma significativa a resiliência e distribuição das mesmas. Por fim, as análises aqui utilizadas se mostraram eficientes, sendo de fato importantes ferramentas para a fisiologia da conservação do grupo.



Palavras-chave: Raias, tubarões, salinidade, temperatura, manejo pesqueiro, fisiologia da conservação.

## ABSTRACT

Top mesopredators are of upmost importance for the health and balance of the ecosystems in which they are inserted. Environmental imbalances that cause ecological disruption primarily affect animals occupying higher trophic levels, being sharks and mammal's important indicators (sentinels) of impacts. Elasmobranchs are constantly exposed to anthropogenic pressure, leading to an expressive reduction of populations worldwide. Among the impacts with direct effects on sharks and batoids, overfishing is the most expressive activity, responsible for declines up to 90% for some species of great ecological importance (e.g. white sharks, hammerhead sharks). In the last decades, the marked decline of top predators has already exhibited negative effects at trophic level, reducing the resilience and ability to *cope* with stress of key species. In addition, the effects of climate change on distribution and survival are already observed, affecting not only the ecosystem balance, but also the economy based on commercial capture. The use of physiological tools as a method to assess plasticity and vulnerability is a promising field, since it allows a deeper understanding of how organisms respond to anthropic stressors and environmental changes. Thus, conservation physiology may help to solve management issues by elucidating basic biological process that were evolutionarily molded and determine how an organism will respond to immediate threats. Based on this premise, this doctoral thesis had as objective to use physiological analyzes to evaluate the mechanisms activated during stressful conditions which these animals are commonly exposed (capture and handling; air exposure) or may be exposed due to climate change (thermal and saline alterations). The first four chapters focus on the deleterious effects of fishing (commercial and recreational) on *Zapteryx brevirostris* (chapter 1), *Galeocerdo cuvier* (chapter 2), *Ginglymostoma cirratum* (chapter 3) and *Carcharhinus limbatus* (chapter 4) using physiological tools (plasma analysis and infrared scanning) to evaluate the mechanisms activated facing stress of capture, handling and air exposure. The final two chapters of the thesis aim to exploit the plasticity of *Zapteryx brevirostris* (Chapter 5) and *Sphyrna mokarran* (Chapter 6) facing environmental alterations predicted by climate changes. Plasma analyzes and infrared scanning were also used. Our results demonstrate that all the stressors analyzed are significant, altering the homeostatic patterns of the species studied here, being able to reduce significantly their resilience and distribution. Finally, the analyzes applied in the present study proved to be efficient, being in fact important tools for conservation physiology.



Key-words: Batoids, sharks, salinity, temperature, fishing management, conservation physiology.

## LISTA DE FIGURAS

CAPÍTULO 1



CAPÍTULO 2

statistical differences ($p<0.05$) between live (n=5) and dead sharks (n=3). A Student's t-Test (paired two-samples) was used for differences between capture and handling, and Student's t-Test (unpaired two-sample comparison) was used for differences between live and dead tiger sharks. Each box represents the values grouped, the line represents the mean, the bar represents the standard deviation and the black points represents the outliers.

**Figure 4.** Osmolality, urea, sodium and chloride concentrations in plasma for live (capture and handling) and dead tiger sharks.The symbol (*) represents statistical differences ($p<0.05$) between live (n=5) and dead sharks (n=3). The symbol (**) denotes statistical differences between capture (n=5) and handling (n=5) in live sharks. A Student's t-Test (paired two-samples) was used for differences between capture and handling, and Student's t-Test (unpaired two-sample comparison) was used for differences between live and dead tiger sharks. Each box represents the values grouped, the line represents the mean, the bar represents the standard deviation and the black points represents the outliers.

CAPÍTULO 3

**Figure 1.** Drumline fishing apparatus: (a) bullet floats; (b) large floating poly-ball; (c) rope attaching float to submerged weight; (d) ~18kg cement weight; (e) hook timer; (f) main line of ~410 kg test monofilament; (g) 4 m double-stranded leader of ~410 kg test monofilament; (h) 16/0 5°-offset circle hook. The distance between (e) and (h) is 23 m, and the length between (a) and (d) ranged from 30 to 60m. (Gallagher *et al.* 2014).

**Figure 2.** Representative infrared images of the dorsal and lateral axis of nurse sharks (A – female 1; B – male 8; C – male 11; D – female 14; see table 1 and 2 for more details) after fight response to experimental capture. The increased flank temperature (°C) at landing can be observed (yellow and green coloration). The thermal scale bar on right shows the temperature gradient.

**Figure 3.** Relation between plasma lactate (mM) and time hooked (minutes) of each animal sampled.

**Figure 4.** Relation between plasma lactate (mM) and final flank temperature (°C) of each animal sampled.

CAPÍTULO 4

**Figure 1.** Thermal images being taken from an adult blacktip during scientific sampling. Photo by Alex Anstett.

**Figure 2.** Representative infrared images of the body from two juveniles (females) *C. limbatus* after landing (T1) and six minutes (T2) of experimental exposure to solar radiation. Body temperature according to the scale bar to the right.

**Figure 3.** Representative infrared images of the body from two juveniles (males) *C. limbatus* after landing (T1) and six minutes (T2) of experimental exposure to solar radiation. Body temperature according to the scale bar to the right.

**Figure 4.** Representative infrared images of the body from two adults (females) *C. limbatus* after landing (T1) and six minutes (T2) of experimental exposure to solar radiation. Body temperature according to the scale bar to the right.

**Figure 5.** Representative infrared images of the body from two adults (males) *C. limbatus* after landing (T1) and six minutes (T2) of experimental exposure to solar radiation. Body temperature according to the scale bar to the right.

**Figure 6.** Representative infrared images body regions of *C. limbatus* taken in intermediate time of exposure ($T_{int}$). The grey arrows indicate the regions were the increase in temperature was faster (flanks) and the white arrows indicate the regions where the increase in temperature was slower (head and fins). Body temperature according to the scale bar to the right.

**Figure 7.** Representative infrared images of teleost fishes air exposed during scientific sampling (~6 minutes). Body temperature according to the scale bar to the right.

CAPÍTULO 5

**Figure 1.** Stomach from a dead specimen dissected to access volume capacity, which is 2ml in the species. Photo by Natascha Wosnick.

**Figure 2.** Testing the syringe size for easy access of the stomach through the animal's mouth. Note that in the photo in the right the stomach was removed to validate the correct positioning of the syringe. Photo by Natascha Wosnick.

**Figure 3.** Plasma osmolality of guitarfishes exposed to different salinities for six (35 and 25 psu) and twelve (25 >35 psu) hours. The green line represents liquid stomach content (water) in guitarfishes exposed to different salinities The lowercase letters represent statistical differences among treatments (one-way ANOVAs, and post hoc test of Dunn's - limit of significance of 0.05).

CAPÍTULO 6

**Figure 1.** Representative infrared images of the body from *S. mokarran* (A) at one minute (T1) and two minutes (T2) of experimental exposure to solar radiation. Infrared images of the cephalofoil from females (B-F) at one minute (T1) and two minutes (T2) of

exposure to solar radiation. The thermal scale bar on right shows the temperature gradient**.**

**Figure 2.** Infrared images of the cephalofoil from males (A-F) at one minute (T1) and two minutes (T2) of experimental exposure to solar radiation. The thermal scale bar on right shows the temperature gradient.

**Figure 3.** Comparison of measured water temperatures at capture versus cephalofoil temperatures at the first minute post exposure to solar radiation (T1) as well as comparisons of measured air temperature versus cephalofoil temperature (T2) at second minute post experimental exposure to solar radiation. Black circles are mean cephalofoil temperatures (°C) ± standard error (wiskers). The dark blue bold line is the mean water temperature (°C) ± standard error (dark blue shaded area). The light blue bold line indicates mean air temperature (°C) ± standard error (light blue shaded area). Student's t-Tests were used to statistically compare water and air temperatures versus cephalofoil temperatures.

**Figure 4.** Representative infrared thermal images of heads from (A) blacknose (*Carcharhinus acronotus*), (B) sandbar (*Carcharhinus plumbeus*), (C) nurse (*Ginglymostoma cirratum*) and (D) blacktip (*Carcharhinus limbatus*) sharks after two minutes of experimental exposure to solar radiation. The thermal scale bar on right shows the temperature gradient.

**Figure 5.** Infrared thermal images of the cephalofoil from two Scalloped hammerhead sharks (*S. lewini*) (A-B) after one (T1) and two minutes (T2) of experimental exposure to solar radiation. The thermal scale bar on right shows the temperature gradient.

**Figure 6.** Thermal accommodation theory. The scheme exhibit the increase in the cephalofoil temperature as an evolutionary ability to properly accommodate the ampullae to an abrupt change in the surrounding temperature when entering in shallow waters, ensuring this way a proper electrical perception during foraging. Scheme by National Geographic on Mega Hammerhead special (SharkFest 2016).

**Figure 7.** Geographical comparison of *S. mokarran* distribution (based on Compagno 2001) versus both sea surface temperatures and solar radiation predictions for 2100, for RCP8.5 (IPCC 2014) and SRES A1B (Bais et al. 2010) scenarios, respectively. The current distribution of *S. mokarran* is identified by dark red contours along coastal shorelines, primarily bound between latitudes 45°N and 35°S. With parallel lines enclose latitudinal areas that will experience predicted reductions in solar radiation in year 2100. The related solar radiation values are provided in the white boxes bound by the associated latitudinal bounding areas demarked by withe parallel lines.

## LISTA DE TABELAS

CAPÍTULO 2



CAPÍTULO 3



CAPÍTULO 4



CAPÍTULO 6

**SUMÁRIO**

## GENERAL INTRODUCTION

Elasmobranchs are unarguably among the most endangered animals in the world, with population declines up to 90% in some regions for some species (Dent and Clarke 2015). It has been estimated that around 25% of the species currently described are facing some level of threat (Dulvy *et al.* 2008), however, it is possible that this estimative is even higher. Due to its life history, sharks and batoids are especially vulnerable to human activities, such as habitat degradation, pollution and overexploitation (Dulvy *et al.* 2008). Currently, about one hundred million sharks are killed annually, mostly for the fin trade (Worm *et al.* 2013). The number for batoids it is currently unknown.

The high level of catches has raised international concern over the sustainability of elasmobranch fisheries, however, most of the countries that overexploit local populations have little or no management plans in place for their shark and batoids resources and almost nothing is known about the status of the fishery stocks (Clarke *et al.* 2006a; Dent and Clarke 2015). Currently, the domestic regulations on exploitation differs by country, making difficult the improvement of management plans for most of the species, since sharks and batoids may exhibit a highly migratory behavior (Compagno 2001; Hammerschlag *et al.* 2011).

The population collapse is an eminent consequence and it is already observed in some countries that strongly relies on elasmobranch fishing. For example, in India the annual catch rate decreased significantly in the past years, affecting the economy and the representativity of the sector in the Gross Domestic Product (Hausfather 2004). Currently, despite the growing capture effort and the improvement of fishing technology, the annual global catch (without taking into account unregulated and unreported catches) is continually decreasing, indicating a strong decline in populations worldwide (Dent and Clarke 2015). Regardless of the catch recorded by FAO, studies on shark biomass from commercial fishing demonstrate that the realistic annual catch may be three to four times higher than the currently reported (Clarke *et al.* 2006b).

Studies focusing on elasmobranch survival rates are now taking place, since most of the Conservation Management Plans at domestic and international level discuss the implementation of compensatory practices, such as immediate

release, as a strategy to reduce the impacts of fishing, at least for species commonly caught as bycatch. It has been estimated that around 95% of the species commonly caught in industrial fishing are still alive when brought on board (Moyes *et al.* 2006), enforcing the efficiency of immediate release and catting attention of researchers, policy makers and wildlife managers.

Allied to the strong pressure caused by human activities, sharks and batoids are now facing several environmental changes related antropic activities. The most immediate and already observed impacts are in the patterns of marine biodiversity through changes in species' distributions. A modeling performed by Cheung *et al.* (2009) showed a significant loss of habitat for several marine species and dramatic species turnovers of about 60% on the current biodiversity, leading to an imminent disruption in ecological processes. For elasmobranchs, which are mostly ectothermic and dependent on the environment temperature for a satisfactory metabolic functioning, alterations in the environmental thermal gradient may lead to changes in their latitudinal and depth ranges (Perry *et al.* 2005; Parmesan 2006; Hiddink and Hofstede 2008; Mueter and Litzow 2008; Dulvy *et al.* 2008). Such changes may lead to niche relocation that may affect lower trophic levels in these modified ecosystems.

Elasmobranch populations are crucial for a healthy and balanced ecosystem. The reduction in its abundance may lead to disruption at trophic level. Many top predators (APEX) also play an important role as scavengers by removing debilitated animals and diseases of prey populations (Heupel *et al.* 2014). Since several species of sharks and batoids are extremely active, exhibiting high rates of Basal Metabolism (BMR) and Oxygen Consumption by Mass (IMR) (Rosa and Seibel 2008; Whitney *et al.* 2016), it is believed that current increase in pollution, the predicted ocean acidification, the reduction of the dissolved oxygen available and increase in water salinity may affect negatively the metabolic rate and swimming capacity of species with great ecological representativity (Chin *et al.* 2010). The immediate implementation of contingency plans is crucial since top predators can help to reduce the effects of climate changes, acting like buffers in marine ecosystems (Sala 2006). Given that the plasticity of non-native species tends to be high, often exceeding the tolerance limits of native species, and that climate change could potentiate this capacity, it is believed that the role of regulators exercised by top predators could

reduce the advance of invasive species through control by active predation (Sala 2006).

Conservation physiology is defined as an integrative scientific discipline which aims to apply physiological tools to characterize biological diversity and its ecological implications (Wikelski and Cooke 2006). The use of physiological tools allows a deeper understanding of how organisms, populations, and ecosystems respond to environmental changes and stressors, thus helping to solve conservation problems across taxa (Cooke *et al.* 2013). It goes beyond a description of patterns, taking into account the understanding of the mechanisms that causes conservation issues (Stevenson *et al.* 2005).

Since the human influence is increasing continuously, a better understanding of which anthropogenic aspects trigger stress responses in animals is imperative to the development of effective countermeasures (Wikelski and Cooke 2006). Not only the type but also the duration of a stressor determines the ability to *cope* of an organism. While acute stress such as fishing tends to last only for a short period of time, chronic stress, such as climate changes are characterized by long-term exposure.

Physiological responses can be used to the assessment of the realistic effects of each stressor and the ability to recover of each species. For example, blood samples allow the assessment and quantification of stressors (acute and chronic) that can affect fitness and survival. In this context, this PhD thesis addressed the physiological effects of fishing and environmental alterations in endangered elasmobranch species, as a way to better understand the impacts that both stressors may have on keystone species and how its vulnerability may affect not only the species, but also the species with which they interact in ecological level. For that, plasma samples and infrared thermography were used to determine the causes and effects of exposure to antropogenic and environmental stressors.

**REFERENCES**

Cheung, W. W., Lam, V. W., Sarmiento, J. L., Kearney, K., Watson, R., Pauly, D. (2009). Projecting global marine biodiversity impacts under climate change scenarios. Fish and Fisheries, 10(3), 235-251.

Chin, A., Kyne, P. M., Walker, T. I., Mcauley, R. O. R. Y. (2010). An integrated risk assessment for climate change: analysing the vulnerability of sharks and rays on Australia's Great Barrier Reef. Global Change Biology, 16(7), 1936-1953.

Clarke, S. C., McAllister, M. K., Milner-Gulland, E. J., Kirkwood, G. P., Michielsens, C. G., Agnew, D. J., ... Shivji, M. S. (2006a). Global estimates of shark catches using trade records from commercial markets. Ecology Letters, 9(10), 1115-1126.

Clarke, S. C., Magnussen, J. E., Abercrombie, D. L., McAllister, M. K., Shivji, M. S. (2006b). Identification of shark species composition and proportion in the Hong Kong shark fin market based on molecular genetics and trade records. Conservation Biology, 20(1), 201-211.

Compagno, L. J. V. (2001). Sharks of The World: An Annotated and Illustrated Catalogue of Shark Species Known to Date. Vol. 2. No. 1. Food Agriculture Org.

Cooke, S. J., Sack, L., Franklin, C. E., Farrell, A. P., Beardall, J., Wikelski, M., Chown, S. L. (2013). What is conservation physiology? Perspectives on an increasingly integrated and essential science. Conservation Physiology, 1(1), 001.

Dent, F., Clarke, S. (2015). State of the global market for shark products. FAO Fisheries and Aquaculture Technical Paper, 590.

Dulvy, N. K., Baum, J. K., Clarke, S., Compagno, L. J., Cortes, E., Domingo, A., ... Martínez, J. (2008). You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. Aquatic Conservation: Marine and Freshwater Ecosystems, 18(5), 459-482.

Hammerschlag, N., Gallagher, A. J., Lazarre, D. M. (2011). A review of shark satellite tagging studies. Journal of Experimental Marine Biology and Ecology, 398(1), 1-8.

Hausfather, Z. (2004). India's shark trade: an analysis of Indian shark landings based on shark fin exports.

Heupel, M. R., Knip, D. M., Simpfendorfer, C. A., Dulvy, N. K. (2014). Sizing up the ecological role of sharks as predators. Marine Ecology Progress Series, 495, 291-298.

Hiddink, J. G., Hofstede, R. T. (2008). Climate induced increases in species richness of marine fishes. Global Change Biology 14, 453–460.

Moyes, C. D., Fragoso, N., Musyl, M. K., Brill, R. W. (2006). Predicting postrelease survival in large pelagic fish. Transactions of the American Fisheries Society, 135(5), 1389-1397.

Mueter, F. J., Litzow, M. A. (2008). Sea ice retreat alters the biogeography of the Bering Sea continental shelf. Ecological Applications 18(2), 309–320.

Parmesan, C. (2006) Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution and systematics 37, 637–669.

Perry, A. L., Low, P.J., Ellis, J.R., Reynolds, J.D. (2005). Climate change and distribution shifts in marine fishes. Science, 308, 1912–1915.

Sala, E. (2006). Top predators provide insurance against climate change. Trends in Ecology and Evolution, 21(9), 479-480.

Stevenson, R. D., Tuberty, S. R., Wingfield, J. C. (2005). Ecophysiology and conservation: the contribution of endocrinology and immunology–introduction to the symposium. Integrative and Comparative Biology, 45(1), 1-3.

Wikelski, M., Cooke, S. J. (2006). Conservation physiology. Trends in Ecology and Evolution, 21(1), 38-46.

Worm, B., Davis, B., Kettemer, L., Ward-Paige, C. A., Chapman, D., Heithaus, M. R., ... Gruber, S. H. (2013). Global catches, exploitation rates, and rebuilding options for sharks. Marine Policy, 40, 194-204.

# CAPÍTULO 1

**Bycatch on the lesser guitarfish, *Zapteryx brevirostris* (Chondrichthyes: Rhinobatidae): survival rates, physiological status and reproductive consequences**

**Abstract**

Globally, sharks and rays are mainly caught as bycatch of fisheries targeting other species. Approximately 50% of elasmobranch global production is composed of bycatch. The fate of bycatch species not used as a byproduct and released back into the water is crucial to understand the population status of the species of elasmbranchs and consequent decision to implement adequate management measurements. The lesser guitarfish, *Zapteryx brevirostris,* is an endemic species of South Atlantic listed as “vulnerable” by the International Union for Conservation of Nature (IUCN) red list. Although, the species has low commercial value and display a high tolerance to capture, being landed alive after surviving out of the water up to 6 hours, a significant reduction of survival rates have been reported to occur during the reproductive period. The aim of this study was to examine post capture abortion consequences and survivability in pregnant female lesser guitarfishes obtained as bycatch from artisanal fisheries in Southern Brazil. Data on plasma urea, phosphorus and potassium from pregnant and non-pregnant females were compared. Results showed high successive abortion rates and reduced survivability; all the females suffered abortions within 48 hours after capture with a post-abortion mortality of 37%. Pregnant females showed a decrease in urea and an increase in lactate in plasma compared with non-pregnant females. These results were attributed to the loss of homeostasis probably caused by increased trauma due to high metabolic demands in pregnant females. From this study, we strongly recommend the implementation of a management plan where the species is immediately release after capture in order to minimize post release mortality during the reproductive period.

## 1 Introduction

Bycatch is traditionally defined as the incidental capture of non-target species or unwanted size ranges of target species through a non-selective fishing that is either unused or unmanaged (Crowder and Murawski 1998; Davies *et al*. 2009). The ‘unused’ term addresses the discarded portion of the catch whilst ‘unmanaged’ refers to the catch for which no specific management measures are clearly defined and directed to achieve sustainability (Davies *et al.* 2009). Thus, any species landed and/or non-retained are considered bycatch when there is no

regulation for their extractions (Oliver *et al.* 2015). Elasmobranchs are frequently caught in commercial fisheries as a bycatch (discarded) or byproduct (retained) resulting in the primary threat to sharks and rays. Due of their life history characteristics as late maturity, long life spans and long incubation/gestation periods, the group is particularly vulnerable to fishing activities (Dulvy *et al.* 2008; Ferretti *et al.* 2008; Dulvy *et al.* 2014).

Although different incidental catch patterns exist for different fishing gears, habitats and species, approximately 50% of the global elasmobranchs production is composed of bycatch counting for 67% of elasmobranch species reported by the IUCN that are facing conservation threats (Molina and Cook 2012; Oliver *et al.* 2015). Much of discards in the bycatch are not documented, which makes a proper assessment difficult to perform (Barker and Schluessel 2005; Dulvy *et al*. 2014). In general, only a small proportion of the bycatch in commercial fisheries survive during the process (Braccini *et al.* 2013; Oliver *et al.* 2015). The specific reporting of batoids bycatch is notoriously poor on a global scale, and improvements in species-specific bycatch statistics are necessary to allow better monitoring of populations and effective management strategies (Stevens *et al.* 2000; Oliver *et al.* 2015).

Thus, detailed reports on bycatch consequences, i.e. mortality rates due the species either being retained or due post release mortality, are necessary in order to assist with the implementation of adequate bycatch management measures as an integral part for conservation, sustainable fisheries and ecosystem monitoring schemes (Hall 1996; Barker and Schluessel 2005). However, even for species that are brought on board and immediately release or even landed alive, compensatory practices may be compromised by the physiological disturbances caused by the capture stress (Skomal 2007; Skomal and Mandelman 2012) leading to a high rate of post-release mortality or sub-lethal effects on their reproductive capacity (Guida *et al.* 2017).

Disturbances on the interactions between the hypothalamus–pituitary–adrenal (HPA) axis and the hypothalamus–pituitary–gonadal axis (HPG), which triggers all aspects of reproduction in vertebrates including elasmobranchs, are among the most consistently negative effects of stress (Wingfield and Sapolsky 2003; Awruch 2013). Thus, individuals caught during the reproductive period are

even more vulnerable to capture and release due to the high metabolic demands towards reproduction and less resources allocated to immediately respond to stress (French *et al.* 2007). As consequences, even minor stress responses may have inherent metabolic costs as the resources allocated to fight stress reduce reproductive investment (Wingfield and Sapolsky 2003; Awruch 2013).

Allied to that, disturbances in the homeostatic profile are closely related to inability to recover and high mortality rates facing stressful situations (Wosnick *et al.* 2016) further reducing the chances of survival of species caught as bycatch. Secondary parameters such as urea, lactate and potassium ($K^+$) are reliable tools for stress studies in elamsobranchs since alterations in plasma concentrations often indicate homeostatic disruption (Brooks *et al.* 2012, Gallagher *et al.* 2014). Furthermore, a new physiological secondary stress parameter, phosphorus ($P^{3+}$), was measured to confirm its use as a stress indicator as previously validated for tiger sharks (Wosnick *et al.* 2016). Phosphorus is involved in cell functioning, membrane formation and phospholipids production and its imbalance can cause heart failure (Neves *et al.* 2004). Changes in phosphorus concentration may be related to stress since its increase may indicate cytolysis, renal failure and gill collapse (Wosnick *et al.* 2016). Despite its close relation with intracellular damage, phosphorus is rarely used as stress indicator.

Viviparous elasmobranchs are potentially vulnerable to reproductive disruptions due to capture stress since their relatively long gestation periods increase the probability of post release mortality or sub-lethal reproductive effects being caused by fishing whilst pregnant (Guida *et al.* 2017). One of the main sub-lethal consequences of stress during the reproductive period is abortion, characterized by the spontaneous expulsion of an embryo before it is sufficiently developed to survive. This type of trauma has already been reported for a number of viviparous elasmobranch species, with involuntary embryo loss at different stages of development as a consequence of capture and handling (Rincon 2007, Zagaglia *et al.* 2011, Schluessel *et al.* 2015). Thus, a better understanding of possible abortion consequences due capture stress is of utmost importance in order to assure the next breeding season and next year recruitment, primary requirements for successful sustainable and conservation management programs.

The lesser guitarfish *Zapteryx brevirostris* (Müller and Henle 1841) is an endemic batoid of the South Atlantic, distributed from eastern Brazil to northeastern Argentina (Figueiredo 1977; Castillo-Páez *et al.* 2017). The species exhibit a lecithotrophic viviparous reproductive mode, with four to eight (average six) embryos per breeding cycle (Batista 1991; Abilhoa *et al.* 2007). The copulatory period occurs during the South Hemisphere winter (June-August), followed by embryonic development in spring (September-November) and birth in summer (December-February) (Batista 1991; Abilhoa *et al.* 2007). *Zapteryx brevirostris* exhibit low commercial value and it is commonly caught as bycatch in shrimp (*Xiphopenaeus kroyeri*) trawl fisheries and gillnetting that target flatfish (*Paralichthyus isosceles, P. Orbignyanus, P. Sosceles, P. patagonicus*) (Costa and Chaves 2006; Bornatowski and Abilhoa 2012), with several animals captured every day and a peak of capture during spring and summer (~100 animals per vessel per day). The species seems to display high tolerance to capture, being landed alive and surviving out of the water up to six hours (field observation). However, consecutive abortions and higher mortality rates are observed in females during pregnancy.

Although the species is discarded, due to declines reported in Argentina, heavy fishing pressure in the center of its abundance in Brazil and low fecundity, the species has been listed as Vulnerable" in the IUCN Red List of Threatened Species (2016) and regarded as Vulnerable (VU) along the Brazilian coast following IUCN criteria (Brasil 2014) but may move to be listed as threatened in the near future as heavy fishing pressure continues or even increases.. In this context, this research will address the effects of bycatch commercial capture and release of pregnant *Z. brevirostris*. The results from this study will provide new information to minimize the impact caused by incidental capture, suggesting capture and handling practices in order to assists with satisfactory management plans for this endemic and endangered species, focusing in pregnant females.

## 2 Material and methods

### 2.1 Animals

Thirty non-pregnant females were obtained during the cold months (June-July 2012, 16 - 18°C water temperature and 7 - 15° C air temperature) and 60

pregnant females during the warmer months (September - November 2014, 21 - 22°C water temperature and 30 - 34°C air temperature). All individuals were caught as bycatch from gillnet artisanal fishery in the state of Paraná, Southern Brazil (Figure 1). Paraná coast strongly relies in small-scale fishing and elasmobranch bycatch represent a significant part of the daily captures in the region.

During the commercial capture, gillnets were kept in the water for 48 hours. Caught females were then transported by fishermen (~ 2 hours) to the fish market where the animals were bought and transferred to the laboratory following transportation protocols established by Smith *et al.* (2004). Briefly, females were transported by car for about 10 min in black plastic boxes (56.4 cm × 38.5 cm × 37.1 cm) with 20 liters of seawater, constant aeration, 32 psu salinity, and 18°C and 28°C water temperature during the cold and warmer months respectively. Quality of the water (temperature, salinity, dissolved oxygen and nitrogen concentration) was monitored before and after transportation, without changes in the parameters. The capture and sampling were approved by the Brazilian Ministry of Environment (IBAMA/ICMBio-SISBIO #20030) and by the Ethics Committee on Animal Use (CEUA-UFPR #776/2014).

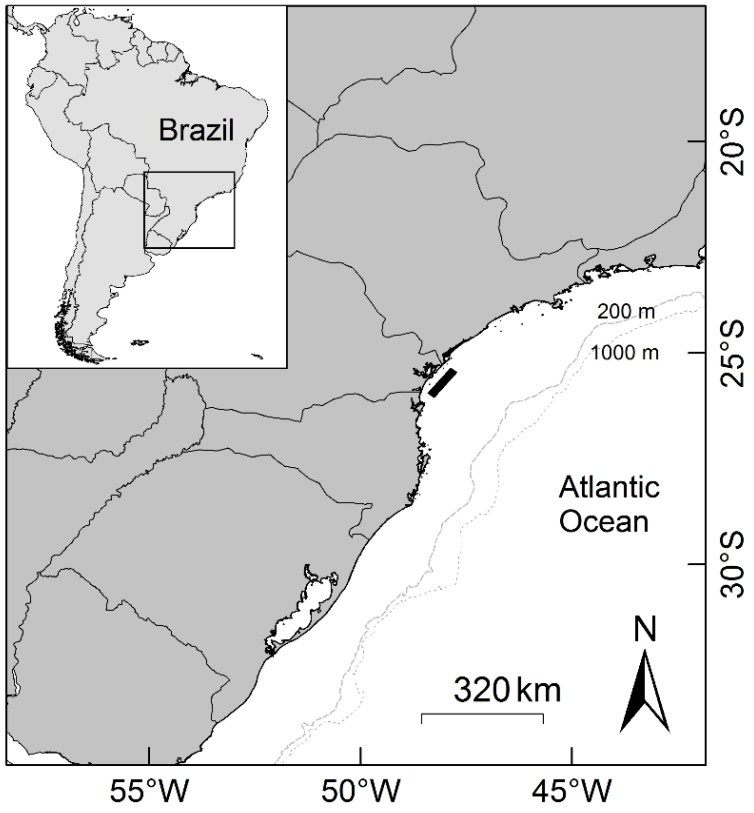


**Figure 1.** Area where the animals were obtained from artisanal fishermen. Paraná coast strongly relies in small-scale fishing and elasmobranch bycatch represent a significant part of the daily captures in the region.

## 2.2 Captivity monitoring

Females were analyzed for possible signs of pregnancy (Conrath 2004). For the diagnosis, the presence of bruises on the cloaca (caused by copulation) and abdomen distension were first evaluated (Figure 2). Once in the laboratory females were first placed for 30 min into temporary plastic boxes (~50 liters) with constantly renewed water, before being transferred to permanent holding tanks of 130 liters of seawater, maintaining same salinity and temperature throughout the experimental period. This first 30 min holding step was necessary to avoid contamination in the permanent holding tanks. Once in these permanent tanks, individuals were monitored every hour for two days to record mortality and abortion rates in pregnant females. Animals were fasted during the monitoring period to avoid changes in plasma parameters used to measure stress.

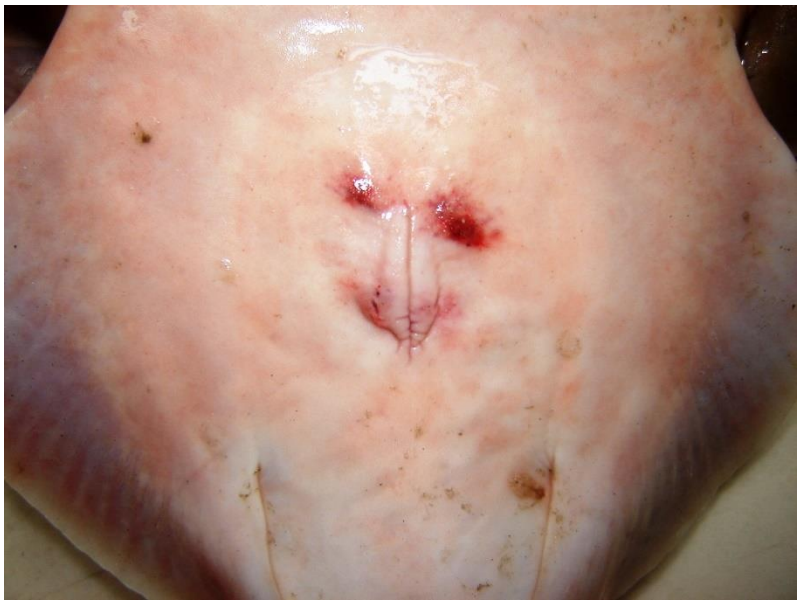


**Figure 2.** Diagnosis of a female *Z. brevirostris* with signs of pregnancy (bruises on the cloaca and abdomen distension) based on description made by Conrath (2004). Photo by Hugo Bornatowski

## 2.3 Plasma assays

To test the physiological conditions of both pregnant and non-pregnant females that were kept in the aboratory under controlled conditions, blood samples (5 ml) from females that died after successive abortions (1 hour after death), females that survived after successive abortions and non-pregnant females were obtained by caudal vein puncture using pre-heparinized syringes fitted with 26.5G needles. After withdrawal, blood samples were immediately centrifuged for seven minutes at room temperature (20°C) at 2000 g. Plasma was separated and kept frozen at -20ºC until analysis at the Laboratory of Comparative Physiology of Osmoregulation in Curitiba, Paraná.

Urea, lactate, concentrations of Phosphorus ($P^{3+}$), Potassium ($K^{+}$), and pH were assayed from plasma samples. Urea (Labtest, Brazil, catalog n. 27; wave-length 600nm), lactate (catalog n. 138-1/50; wave-length 550nm) and phosphorus (catalog n. 42; wave-length 650nm) levels were quantified colorimetrically (Ultrospec 2100 PRO Amersham Pharmacia biotech, Sweden). Potassium was quantified using flame photometry (CELM FC-180, Brazil). For the urea and potassium analyses, plasma samples were diluted in ultrapure water (urea - 1:50; potassium – 1:200). For pH measurements, the pH meter (inoLab, Level 1, WIW, Germany) was calibrated using buffers (Neon, Brazil) of pH 4.0, 6.86 and 9.18. After calibration, the samples were read at room temperature (20°C).

## 2.4 Statistical analysis

To verify if capture stress induces abortion, the number of abortions experienced by each female were recorded and fitted to a non-linear regression. Consecutives abortions were defined when females aborted more than once within the monitored period. Spearman rank correlation was used to verify the relationship between number of pups aborted by each female and time after capture. For the assessment of the stress caused by capture and consecutive abortions, one-way ANOVA test was used to assess differences in the physiological variables concentrations (urea, lactate, $P^{3+}$, $K^{+}$ and pH) between non-pregnant, pregnant that suffer consecutive abortions and survived and pregnant that died after consecutive abortions. The normality was verified using Shapiro-Wilk and homogeneity was verified using Levene's test. To assess which

groups were different a Tukey test was used. All tests were performed with a limit of significance of 0.05, using R software for statistical computing and graphics (R Development Core Team, 2016).

## 3 Results

No mortality was registered for the 30 non-pregnant females monitored within 48 hours after landing. Regarding the pregnant females (n=60), 25% died during transport (n=15) and 60% died after consecutive abortions (n=27). Of the total number of pregnant females which survived to transport and were monitored in the laboratory (n=45), 93% suffered consecutive abortions in the first 22 hours of monitoring, with 39% of the total embryos aborted in the first 9 hours (n=80) (Figure 3). The number of abortions was higher soon after landed to decline with time, observing a negative correlation between the number of abortions and the time after landing (Figure 3). An average of 4.5±1.2 embryos were aborted by each female within 48 hours of monitoring (Figure 4).

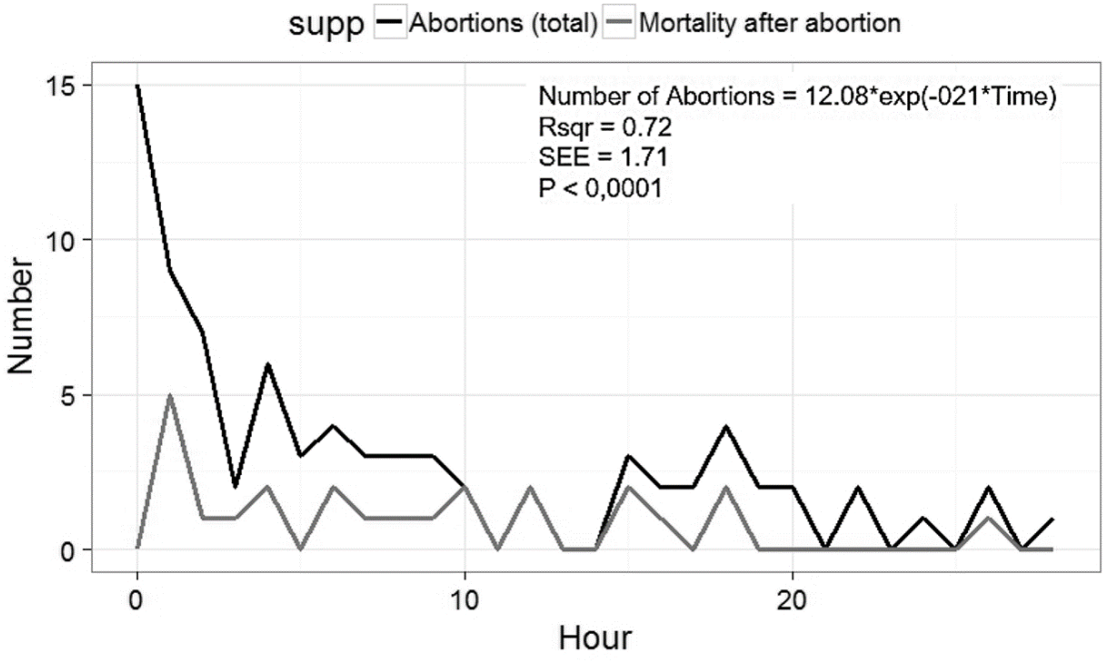



**Figure 3.** Number of abortions (black line) and post-abortion mortality (grey line) in *Zapteryx brevirostris* females kept in laboratory within 48 hours. Total number of pregnant females = 45. The last abortion occurred 26 hours after capture.

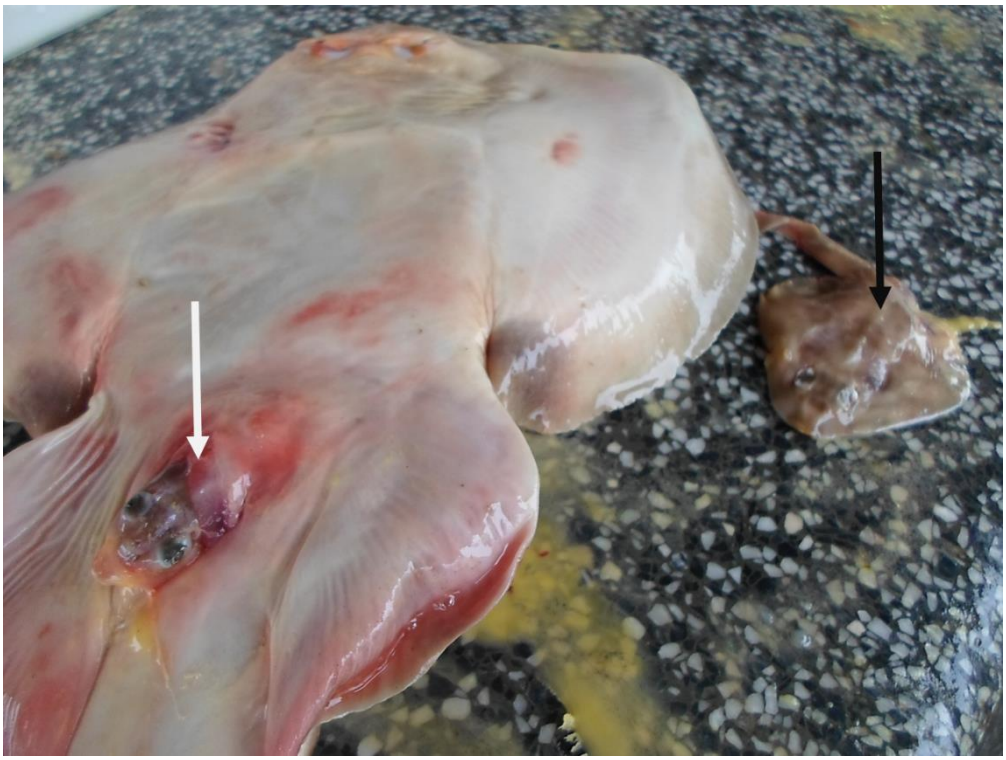


**Figure 4.** Dead female after abortion of two embryos. The black arrow indicates the embryo aborted while she was alive and the white arrow indicates the one that was being aborted when the female ceased the respiration and the death was declared. The reduction of abdomen distension can be noted. Photo by Isabella Simões.

To reduce handling stress that could lead to an even greater increase in the mortality of the monitored animals (Skomal 2007; Skomal and Mandelman 2012), only 6 females that survived after consecutive abortions had their blood collected. For statistical reasons, the same sample size was then established for both dead and non-pregnant females with 100% of survival, totaling 18 animals ramdomly sampled. Regarding the physiological parameters to assess stress, although the number of females sampled were small, important information was obtained from these indicators. Urea plasma concentration, an indicator of homeostatic disruption caused by stress (Wosnick *et al.* 2016), was significant lower in females that died after consecutive abortions (274±6.73 S.E.) compared to the ones surviving after consecutive abortions (380±7.5 S.E.) and non-pregnant females (435±9.8 S.E.) (One-Way ANOVA; F= 101.126 P=<0.001) (Figure 5a).

Similar pattern was observed in plasma pH, a reliable indicator of loss of acid-base homeostasis, being significantly lower in dead pregnant females (5.82±0.124 S.E.) when compared to females that survived after consecutive

abortions (6.79±0.034 S.E.) and non-pregnant females (6.97±0.039 S.E.) (One-Way ANOVA; H = 14.363 P=<0.001) (Figure 5a). Plasma lactate concentrations, which indicates the accumulation of muscle lactate formed during anaerobiosis released to the blood (Skomal 2007), showed an opposite pattern, being significantly higher in females that died after consecutive abortions (47.7±4.46 S.E.) in comparison to pregnant females that survived after consecutive abortions (15±1.12 S.E.) and non-pregnant females (8.8±0.38 S.E.) (One-Way ANOVA; H=15.174 P=<0.001) (Figure 5a).

Plasma $P_3^+$ concentrations indicating cytolysis and loss of intracellular homeostatic capacity (Andriguetto *et al.* 1990) and $K^+$ levels, an indicator of myocardial function and muscle tetany (Hoffmayer and Parsons 2001), were higher in dead pregnant animals ($P_3^+$: 4.57±0.52 S.E.; $K^+$: 37.14± 2.25 S.E.) when compared to live pregnant and non-pregnant females (Figure 5b) (One-Way ANOVA; P=0.002). No statistical differences were distinguished between live pregnant females ($P_3^+$: 0.132±0.002 S.E.; $K^+$: 8.56±0.28 S.E.) and non-pregnant ($P_3^+$: 0.145±0.010 S.E.; $K^+$: 9.17±0.307 S.E.) (Figure 5b) (One-Way ANOVA; P = 0.093 and P = 0.180, respectively).

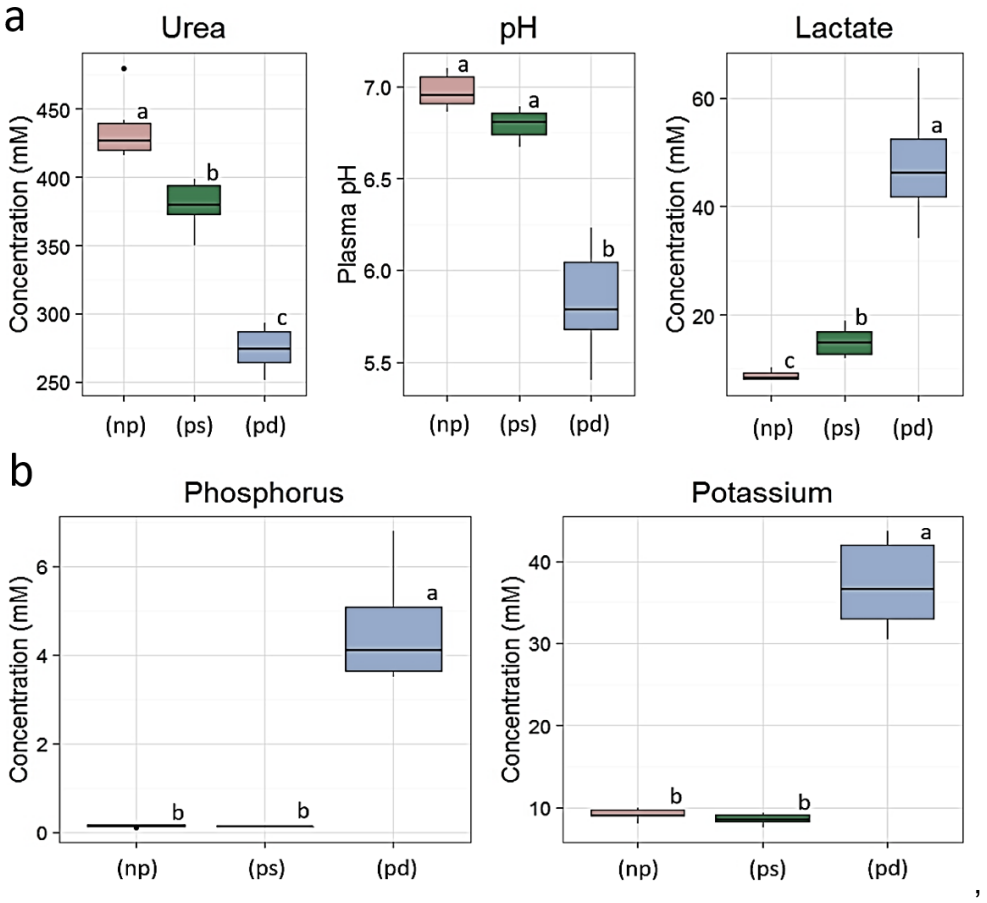



**Figure 5.** Physiological indicators in *Zapteryx brevirostris*. **(a)** Plasma urea, pH and lactate and **(b)** Plasma phosphorus and potassium. Non-pregnant (np) (n=6), females that survived after consecutive abortions (ps) (n=6), females that died after consecutive abortions (pd) (n=6). Each box represents the values grouped, the line represents the mean, the bar represents the standard deviation and the black points represents the outliers. Statistical differences are represented by lowercase letters.

## 4 Discussion

Our results showed that the incidental capture during gestation and birth periods (spring and summer) resulted in higher mortality of females (60%). The high mortality of females captured during the embryonic development is probably related to the fragile state of the animals at this stage of life. However, it is also possible that the higher air temperature during the period that the animals stayed out of the water had affected its physical and physiological integrity, reducing their resilience. The same pattern was observed for the little skate, *Leucoraja eriancea*, with higher mortality after air exposure during summer (100%) when compared to winter (27%) (Cicia *et al.* 2012). That way, it is possible to infer from

the comparison between seasons that the incidental capture during the mating period (winter) may increase the chances of success in compensatory practices (immediate release), while incidental capture during gestation period may reduce drastically the efficiency of compensatory plans, being necessary the improvement of the protocols in this period. Additionally, since most of the commercial discards are composed by a significant amount of parental biomass, management plans need to take into account the mortality rate and post-release survival of pregnant females and sexually active males, in order to properly access the ecological impacts of uninterrupted fishing (Musyl *et al.* 2011).

The high abortion ratio in the first hours observed here may be a strong indicator that the first day after capture is critical and determines the female's recovery. It shows that acute stress (fast exposure - minutes to hours - to one or more stressors) may reduce the chances of pregnancy maintenance and as consequence, the female's survival. The comparison between live and dead females demonstrated that plasma urea, pH, lactate, potassium and phosphorus showed significant alterations. Since all the markers tested are reliable tools to access stress in elasmobranchs (Brooks *et al.* 2012; Mandelman and Farrington 2007; Gallagher *et al.* 2014; Wosnick *et al.* 2016), it is plausible to infer that in fact the females that died after successive abortions showed loss of plasma osmotic/metabolic homeostatic balance as a physiological response to the stress caused by capture and abortions suffered.

Since *post-mortem* analysis can be used as a negative control and when compared to live animals can indicate when parameters are reaching alarming levels that may seriously compromise the ability to recovery (Wosnick *et al.* 2016), the comparison between females that survived to successive abortion with females that died after the trauma, may help to elucidate some of the physiological paths that are depleted during the stress caused by commercial capture/abortion, improving that way compensatory release protocols by reducing the mortality during the embryonic development season. The control group used here (non-pregant females) was captured by the same fishing method but in a different season, that way, we conclude that not only the fishing methodology or fishing gear/time hooked are responsible for the stress that may reduce the post-release survival, but also the environmental conditions (e.g. air

temperature) and the life stage of the animals incidentally captured. So, for a better understanding of the mortality or reduced recovery caused by commercial fishing, it is imperative to take into account those variables.

The females that survived after successive abortions showed lower values of urea and higher values of lactate when compared to non-pregnant females. The reduction in urea concentrations it is compatible with a secondary stress response caused by an external stressor. However, since *Z. brevirostris* is a partially euryhaline species (Wosnick and Freire 2013) that have the ability to switch ionic and osmotic regulatory strategies, the decrease in urea concentration can be reversed, leading to a quick recovery of regular concentrations. The increase in lactate levels is expected during capture as a physiological response to fight, supported by anaerobic metabolism in white muscle (Hoffmayer and Parsons 2001). It is expected that the stress caused by capture or by the physical trauma (abortion) have increased plasma lactate levels and as consequence plasma acidosis, resulting in a total loss of acid-basic balance (Wood *et al.* 1983; Bonga 1997; Thomas *et al.* 1999). Nevertheless, the plasma pH in the females that survived after successive abortions did not differ from the non-pregnant females, leading us to believe that the increase in plasma lactate observed was not deleterious enough to cause a drop on plasma pH, preventing this way plasma acidosis and ensuring the recovery observed.

## 5 Conclusion

Since the lesser guitarfish has no representative commercial value (Vooren *et al.* 2006) and exhibit a high post-capture (100% during winter and 67% during summer) we strongly believe that the immediate release of the species as a compensatory practice would be of great impact on population recovery. This can be a simple and effective practice to reduce their catches. Even with a high abortion ratio after the stress caused by capture, the survivability of the females is relatively high. That way, a simple measure such as the immediate release of the animals may be an effective and sustainable tool to reduce the impact that artisanal fishing has on the populations this, ensuring that way the active participation of artisanal fishermen in the protection of an endemic and endangered species currently not protected by national legislation.

## Literature cited

Abilhoa, V., Bornatowski, H. Freitas, M.O. (2007). Some information on reproduction and embryonic development of the lesser guitarfish *Zapteryx brevirostris* in Southern Brazil. Acta Adriatica, 48, 89–94.

Andrigueto, J. M., Peryl, L., Minardi, I. (1990). Nutrição animal: bases e fundamentos da nutrição animal. Vol. I. Nobel 1, Brazil 396pp.

Awruch, C. A. (2013). Reproductive endocrinology in chondrichthyans: the present and the future. General and Comparative Endocrinology, 192, 60-70.

Batista, V. S. (1991). Aspectos quantitativos da fecundidade e do desenvolvimento embrionário da raia *Zapteryx brevirostris* Müller and Henle 1841 (Pisces, Rhinobatidae) da Enseada de Itaipu, Niterói, Rio de Janeiro. Revista Brasileira de Biologia, 51(3), 495-501.

Barker, M. J., Schluessel, V. (2005). Managing global shark fisheries: suggestions for prioritizing management strategies. Aquatic Conservation, 15, 325-347.

Braccini, M., McAuley, R., Rowland, F. (2013). Temperate demersal gillnet and demersal longline fisheries status report, p. 271–284. In: Status reports of the fisheries and aquatic resources of Western Australia 2012/13: The state of fisheries. W. J. Fletcher, and K. Santoro (eds). Department of Fisheries, Western Australia.

Brasil. MMA. ICMBIO. Portaria nº 445, de 17 de dezembro de 2014. "Lista Nacional Oficial de Espécies da Fauna Ameaçadas de Extinção - Peixes e Invertebrados Aquáticos". <http://www.icmbio.gov.br/portal/biodiversidade/fauna-brasileira/2741-lista-de-especies-ameacadas-saiba-mais.html>. 2014

Brooks, E. J., Mandelman, J. W., Sloman, K. A., Liss, S., Danylchuk, A. J., Cooke, S. J., Skomal, G. B., Philipp, D. P., Sims, D. W., Suski, C. D. (2012). The physiological response of the Caribbean reef shark (*Carcharhinus perezi*) to longline capture. Comparative Biochemistry and Physiology (A), 162, 94–100.

Bonga, S. W. (1997). The stress response in fish. Physiological Reviews, 77(3), 591-625.

Bornatowski, H., Abilhoa, V. (2012). Tubarões e raias capturados pela pesca artesanal no Paraná: guia de identificação. Hori Cadernos Técnicos, (4).

Castillo-Páez, A., Sandoval-Castillo, J., Corro-Espinosa, D., Tovar-Ávila, J., Blanco-Parra, M. D. P., Saavedra-Sotelo, N. C., ... & Rocha-Olivares, A. (2017). Cutting through the Gordian knot: unravelling morphological, molecular, and biogeographical patterns in the genus Zapteryx (guitarfish) from the Mexican Pacific. ICES Journal of Marine Science, fsx021.

Cicia, A. M., Schlenker, L. S., Sulikowski, J. A., Mandelman, W. (2012). Seasonal variations in the physiological stress response to discrete bouts of aerial exposure in the little skate, *Leucoraja erinacea*. Comparative Biochemistry and Physiology A, 162,130-138.

Conrath, C.L. (2004). Reproductive biology. In: Elasmobranch fisheries management techniques. Musick JA, Benfil R (eds) APEC Fisheries Working Group, pp. 133–164

Costa, L., Chaves, P. D. T. D. C. (2006). Elasmobrânquios capturados pela pesca artesanal na costa sul do Paraná e norte de Santa Catarina, Brasil. Biota Neotropica, 6(3), 1-10.

Crowder, L. B., Murawski, S. A. (1998). Fisheries bycatch: implications for management. Fisheries, 23(6), 8-17.

Davies, R. W. D., S. J. Cripps, Nickson, A., Porter. G. (2009). Defining and estimating global marine fisheries bycatch. Marine Policy, 33, 661-72.

Dent, F., Clarke, S. (2015). State of the global market for shark products. FAO Fisheries and Aquaculture Technical Paper, 590.

Dulvy, N. K., Baum, J. K., Clarke, S., Compagno, L. J., Cortes, E., Domingo, A., ... Martínez, J. (2008). You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. Aquatic Conservation: Marine and Freshwater Ecosystems, 18(5), 459-482.

Dulvy, N. K., Fowler, S. L., Musick, J. A., Cavanagh, R. D., Kyne, P. M., Harrison, L. R., ... Pollock, C. M. (2014). Extinction risk and conservation of the world's sharks and rays. Elife, 3, e00590.

Ferretti, F., Myers, R. A., Serena, F., Lotze, H. K. (2008). Loss of large predatory sharks from the Mediterranean Sea. Conservation Biology, 22(4), 952-964.

Figueiredo J. L. (1997). Manual de peixes marinhos do sudeste do Brasil. I. Introdução: Cações, raias e quimeras. São Paulo: Museu de Zoologia da Universidade de São Paulo

French, S. S., DeNardo, D. F., Moore, M. C. (2007). Trade-Offs between the Reproductive and Immune Systems: Facultative Responses to Resources or Obligate Responses to Reproduction?. The American Naturalist, 170(1), 79-89.

Gallagher, A., Serafy, J., Cooke, S., Hammerschlag, N. (2014). Physiological stress response, reflex impairment, and survival of five sympatric shark species following experimental capture and release. Marine Ecology Progress Series, 496, 207-218.

Guida, L., Awruch, C., Walker, T. I., & Reina, R. D. (2017). Prenatal stress from trawl capture affects mothers and neonates: a case study using the southern fiddler ray (*Trygonorrhina dumerilii*). Scientific Reports, 7.

Hall, M. (1996). On bycatches. Reviews in Fish Biology and Fisheries, 6, 319-352.

Hoffmayer, E.R., Parsons, G.R. (2001). The physiological response to capture and handling stress in the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*. Fish Physiology and Biochemistry, 25, 277–285.

Molina, J. M., Cooke, S. (2012). Trends in shark by catch research: current status and research needs. Reviews in Fish Biology and Fisheries, 22, 719-37.

Musyl, M. K., Brill, R. W., Curran, D. S., Fragoso, N. M., McNaughton, L. M., Nielsen, A., ... Moyes, C. D. (2011). Post release survival, vertical and horizontal movements, and thermal habitats of five species of pelagic sharks in the central Pacific Ocean. Fishery Bulletin, 109(4), 341-368.

Neves, K. R., Graciolli, F. G., dos Reis, L. M., Pasqualucci, C. A., Moyses, R. M., Jorgetti, V. (2004). Adverse effects of hyperphosphatemia on myocardial hypertrophy, renal function, and bone in rats with renal failure. Kidney International, 66(6), 2237-2244.

Oliver, S., M. Braccini, M., Newman, S. J., Harvey, E. S. (2015). Global patterns in the bycatch of sharks and rays. Marine Policy, 54, 86-97.

Rincon, G. (2007). A record of abortion in the school shark *Galeorhinus galeus* (Carcharhiniformes, Triakidae) captured on the continental shelf off southern Brazil. Pan-american Journal of Aquatic Science, 2, 53-54.

Schluessel, V., Giles, J., Kyne, P. M. (2015). Notes on female reproductive biology and embryos of the brown guitarfish *Rhinobatos schlegelii* from the Penghu Islands, Taiwan. Ichthyology Research, 62, 342-357.

Skomal, G. B. (2007). Evaluating the physiological and physical consequences of capture on post-release survivorship in large pelagic fishes. Fisheries Management and Ecology, 14, 81-89.

Skomal, G. B., Mandelman, J. W. (2012). The physiological response to anthropogenic stressors in marine elasmobranch fishes: a review with a focus on the secondary response. Comparative Biochemistry and Physiology (A) 162, 146–155.

Smith, M. F. (1992). Capture and transportation of elasmobranchs, with emphasis on the grey nurse shark (*Carcharias taurus*). Marine and Freshwater Research, 43, 325-343.

Smith, M. D., Thoney, W. D., R. Hueter, R. (2004). The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives. Columbus, OH: Special Publication of the Ohio Biological Survey.

Stevens, J., Bonfil, R., Dulvy, N. K., Walker, P. A. (2000). The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. ICES Journal of Marine Science, 57, 476-94.

Thomas, P. M., Pankhurst, N. W., Bremner, H. A. (1999). The effect of stress and exercise on post-mortem biochemistry of Atlantic salmon and rainbow trout. Journal of Fish Biology, 54(6), 1177-1196.

Vooren, C.M., Lamónaca, A.F., Massa, A. & Hozbor, N. (2006). *Zapteryx brevirostris*. The IUCN Red List of Threatened Species 2006: e.T61419A12478303.

http://dx.doi.org/10.2305/IUCN.UK.2006.RLTS.T61419A12478303.en. Downloaded on 29 June 2017.

Wingfield, J. C., Sapolsky, R. M. (2003). Reproduction and resistance to stress: when and how. Journal of Neuroendocrinology, 15(8), 711-724.

Wood, C. M., Turner, J. D., Graham, M. S. (1983). Why do fish die after severe exercise? Journal of Fish Biology, 22, 189-201.

Wosnick, N., Bornatowski, H., Ferraz, C., Afonso, A., Rangel, B. S., Hazin, F. H. V., Freire, C. A. (2016). Talking to the dead: using Post-mortem data in the assessment of stress in tiger sharks (*Galeocerdo cuvier*) (Péron and Lesueur, 1822). Fish Physiology and Biochemistry, 1-14.

Wosnick, N., Freire, C. A. (2013). Some euryhalinity may be more common than expected in marine elasmobranchs: The example of the South American skate *Zapteryx brevirostris* (Elasmobranchii, Rajiformes, Rhinobatidae). Comparative Biochemistry and Physiology A, 166, 36-43.

Wosnick, N., Bornatowski, H., Ferraz, C., Afonso, A., Rangel, B. S., Hazin, F. H. V., & Freire, C. A. (2017). Talking to the dead: using Post-mortem data in the assessment of stress in tiger sharks (*Galeocerdo cuvier*)(Péron and Lesueur, 1822). Fish physiology and biochemistry, 43(1), 165-178.

Zagaglia, C. R., Damiano, C., Hazin, F. H. V., M. K. Broadhurst, M. K. (2011). Reproduction in *Mustelus canis* (Chondrichthyes: Triakidae) from an unexploited population off northern Brazil. Journal of Applied Ichthyology, 27, 25-29.

# CAPÍTULO 2

**The use of *Post-mortem* data in the assessment of stress in Tiger sharks (*Galeocerdo cuvier*) (Péron and Lesueur, 1822)**

**Abstract**

Sharks are very sensitive to stress and prone to a high mortality rate after capture. Since approximately 50 million of sharks are caught as bycatch every year, and current recommendations to reduce the impact of commercial fishing strongly support immediate release, it is imperative to better understand post-release mortality caused by the stress of capture and handling. Blood samples allow the assessment of stress levels which are valuable tools to reduce mortality in commercial recreational and scientific fishing, being essential for the improvement of those conservation measures. Biochemical analyses are widely used for sharks as stress indicators, with secondary plasma parameters (lactate, glucose and ions) being the most often employed assays. However, it is virtually impossible to determine baseline plasma parameters in free ranging sharks, since blood withdrawal involves animal capture and restrain, which are stressful procedures. This study aims at analyzing secondary parameters of five healthy tiger sharks captured with circular hooks and handlines in Fernando de Noronha Islands (Northeastern Brazil), and comparing them with secondary parameters of three dead tiger sharks caught off Recife (also Northeastern Brazil). The results show that the analysis of some plasma constituents in dead animals may be an efficient tool to assess stress and lethality. The results showed that traditional parameters, such as glucose and calcium, needs to be used with caution. The results also demonstrated the extreme importance of urea and phosphorus for assessing stress response and mortality in tiger sharks, both parameters frequently neglected and of utmost importance for shark's homeostasis.

## 1 Introduction

Sharks are extremely sensitive to human activities, especially interactions with fishing (commercial and recreational), which expose them to a variety of stressors, causing physiological and behavioral changes that can seriously compromise their survival (Skomal 2007). Overfishing is the primary threat to elasmobranchs, and approximately 50% of the sharks caught annually are part of bycatch (Dulvy *et al*. 2008), being traditionally defined as incidental capture of non-target species or unwanted size ranges of target species through a non-selective fishing process (Crowder and Murawski, 1998; Davies *et al.* 2009). For

species that are brought on board alive, air exposure combined with the stress of capture can reduce fitness and survival, decreasing the efficiency of compensatory practices, such as immediate release, currently proposed as a way to reduce bycatch impacts (FAO 2011).

Primary blood parameters such as catecholamines and corticosteroids are widely used as stress markers for vertebrates, with the role of cortisol well established for mammals, birds and teleost fish (e.g., Wingfield and Romero 2001). However, for elasmobranchs, the use of primary stress parameters has proven to be an additional challenge since, unlike teleosts, elasmobranchs do not produce cortisol (Skomal and Bernal 2010; Anderson 2012). In sharks and rays the functional corticosteroid expressed is 1α-hydroxycorticosterone. This glucocorticoid was first described by Idler and Truscott (1966). However, the unique chemical nature of 1α-hydroxycorticosterone brings distinct disadvantages to its detection, mostly because it is difficult to synthesize (Anderson 2012) (besides being currently unavailable since is not being produced in any laboratory), thus limiting its use as a stress marker. Some studies employed 1α-hydroxycorticosterone to access its secretory dynamics (Hazon and Henderson 1984) and the effects of dietary protein restriction on this corticoid (Armour *et al.* 1993). Other studies used corticosterone (CS) analysis to access the reproductive status (Rasmussen and Gruber 1993; Manire et al. 2007), maturity (Rasmussen and Crow 1993) and stress response in sharks (Manire *et al.* 2007; Hoffmayer *et al.* 2012) as an alternative to 1α-hydroxycorticosterone. However, since CS corresponds to only 10% of the glucocorticoid levels in elasmobranchs, and none of the published papers was able to truly prove the role of CS in the stress response, its use is still controversial.

Traditionally, secondary parameters such as glucose and lactate have been commonly used as tools for stress analysis in sharks (Cliff and Thurman 1984; Hoffmayer and Parsons 2001; Mandelman and Farrington 2007; Mandelman and Skomal 2009; Marshall *et al.* 2012; Gallagher *et al.* 2014). Plasma ion analysis has also been used and suggested as reliable stress markers for free ranging and captive sharks. Potassium, for instance has its increased levels related to stress response (Wells et al. 1986; Manire *et al.* 2001; Moyes *et al.* 2006; Mandelman and Farrington 2007; Brooks *et al.* 2012).

Regardless of which physiological parameters are used for assessing the stress of capture and handling, baseline parameters of non-stressed animals have always proved to be elusive, due to the impossibility of obtaining them from free ranging animals without any level of stress. Some alternatives have been proposed, such as the use of captive animals (Mandelman and Skomal 2009), reduced time of capture (Marshall *et al.* 2012), behavioral analysis (swimming capacity) (Manire *et al.* 2001; Skomal *et al.* 2007; Hyatt *et al.* 2016), and mathematical modeling (Skomal 2006). However, none of them was proven to be fully effective. The problem of a lack of baseline parameters is that stress studies exhibit a significant amount of data but without control groups. For that reason, most of the discussion about stress in sharks ended up describing numerical results, such as increase or decrease in ion concentrations in plasma, without clear conclusions about if the animals were in fact stressed.

In this context, the aim of this study was to analyze and propose the use of *Post-mortem* data as putative “control/reference” groups. While baseline data of healthy unstressed individuals is challenging to obtain, the use of negative data (lethal) may be a novel and useful control tool. Blood analyses of dead animals can help to establish ranges or limits of values that can indicate when animals are reaching potentially alarming conditions, improving that way compensatory release protocols. This alternative “relationship to reference values” would mean that, instead of “the closer to basal control levels, the less stressed”, we would have “the closer to dead-reference levels, the more stressed and vulnerable”. To validate this new approach, data from tiger sharks that died in longlines were compared to data from healthy tiger sharks caught, tagged and released alive and in good condition.

## 2 Material and methods

### 2.1 Live sharks capture and containment

Five tiger sharks were caught in Fernando de Noronha, Pernambuco, Brazil (3° 51′ 13.71″ S, 32° 25′ 25.63″ W) in July 2014. Capture was performed individually from a small vessel using baited handlines equipped with circle hooks about the 20-m isobath. After the capture, sharks were conducted to the M/V OCEARCH and placed on a hydraulic lift to remove them out of the water for

sampling and tagging. Eye coverage and pump insertion with constant water flow in the animal's mouth were used to help reduce the stress (Smith *et al.* 2004) (Figure 1). Sex, life stage and size (total length – cm) are presented in Table 1.

**Figure 1.** Juvenile tiger shark being contained for sampling following methodology established to reduce the stress of capture and handling. Photo by Robert Snow.

## 2.2 Live sharks blood sampling

The first blood sampling (capture group) was taken immediately after the animal was securely restrained (Mandelman and Skomal 2009), occurring approximately 10 min after capture and 2 min after lifting. The second blood sample (handling group) was obtained after tagging to allow the appraisal of the stress caused by sampling and time out of the water. All procedures were performed within approximately 14 min, avoiding prolonged air exposure. The capture and sampling of live animals were approved by the Ethics Committee on Animal Use (CEUA) 23082.025519/2014 – UFRPE.

### 2.2.1 Dead sharks blood sampling

Tiger sharks were caught by the R/V *Sinuelo*, a boat operating a shark control program in Recife, Pernambuco, Brazil (see Hazin and Afonso 2013, for details). Blood from three dead tiger sharks caught with longlines equipped with baited 17/0 circle hooks were used as *Post-mortem* data. Average soak time of the longline gear was about twelve hours, but the exact time the animal was caught could not be precisely determined. Sex, life stage and size (total length – cm) are presented in Table 1. Blood samples were collected immediately after hauling the animal on board. Blood analysis and dissection were carried out, in order to access the cause of death, all animals were healthy before capture. The reduced sample size reflects the new practices employed here, aimed at reducing lethal sampling of apex predators for scientific purposes (animals accidentally died). The capture and sampling of dead animals were approved by the Brazilian Ministry of Environment (permit number 15083-8) and by the Ethics Committee on Animal Use (CEUA) 23082.003679/2009 – UFRPE.

| Tiger sharks | Sex | Stage | TL (cm) |
|---|---|---|---|
| Live 1 | Female | Juvenile | 259 |
| Live 2 | Male | Juvenile | 182 |
| Live 3 | Male | Juvenile | 204 |
| Live 4 | Male | Juvenile | 244 |
| Live 5 | Female | Juvenile | 243 |
| Dead 1 | Female | Juvenile | 105 |
| Dead 2 | Female | Juvenile | 144 |
| Dead 3 | Male | Juvenile | 284 |

**Table 1.** Sex, life stage and size (total length – cm) of live and dead tiger sharks analyzed in the present study.

### 2.3 Plasma assays

After blood withdrawal, the sample (~5 mL) was immediately centrifuged for 7 minutes at room temperature (20°C). Plasma was separated and kept frozen at -20ºC until analysis at the Laboratory of Comparative Physiology of Osmoregulation in Curitiba, Paraná. Osmolality, urea, total proteins, glucose, lactate and ionic concentrations ($Na^+$, $Cl^-$, $K^+$, $Mg^{2+}$, $Ca^{2+}$, $P^{3+}$) were assayed in

plasma samples. Osmolality was determined using a vapor pressure osmometer (VAPRO 5520, Wescor, USA) in undiluted samples. Urea (Labtest, Brazil, catalog n. 27; wave-length 600nm), total proteins (catalog n. 99; wave-length 545nm), glucose (catalog n. 133-1/500; wave-length 505 nm), lactate (catalog n. 138-1/50; wave-length 550 nm), chloride (catalog n. 49; wave-length 470 nm), calcium (catalog n. 90; wave-length 570 nm), phosphorus (catalog n. 42; wave-length 650 nm) and magnesium (catalog n. 50; wave-length 505 nm) levels were quantified colorimetrically (Ultrospec 2100 PRO Amersham Pharmacia biotech, Sweden). Plasma samples were appropriately diluted in ultrapure water: 1:50 for urea, 1:2 for chloride, and 1:15 for magnesium. Sodium and potassium were quantified using flame photometry (CELM FC-180, Brazil) in samples diluted (1:200) in ultrapure water. For pH measurements, the pH meter (inoLab, Level 1, WIW, Germany) was calibrated using buffers (Neon, Brazil) of pH 4.0, 6.86 and 9.18. After calibration, the samples were read in room temperature (20°C).

### 2.4 Statistical Analysis

Data met assumptions of normality and homogeneity, so a Student's t-Test (paired two-samples) was used to assess differences between plasma parameters in live sharks, comparing values for each same shark after capture and after handling in the boat. A Student's t-Test (unpaired two-sample comparison) was applied to verify differences between independent live and dead sharks. All tests were performed with a limit of significance of 0.05, using R software for statistical computing and graphics (R Development Core Team, 2016).

## 3 Results

The mean pH for live sharks after capture was 6.85±0.21 (S.E) and 6.82±0.24 (S.E) after handling (Figure 1). Glucose, lactate and potassium within live sharks (after capture vs. after handling) showed no significant statistical differences (Figure 2).

For dead animals, pH showed a decrease to 5.71±0.57 (S.E) when compared to live animals (Figure 1). Glucose, lactate and potassium showed statistical differences between dead and live sharks. Glucose showed wide variation among animals, without a clear pattern. Plasma lactate and potassium showed a sharp increase in dead animals (Figure 2).

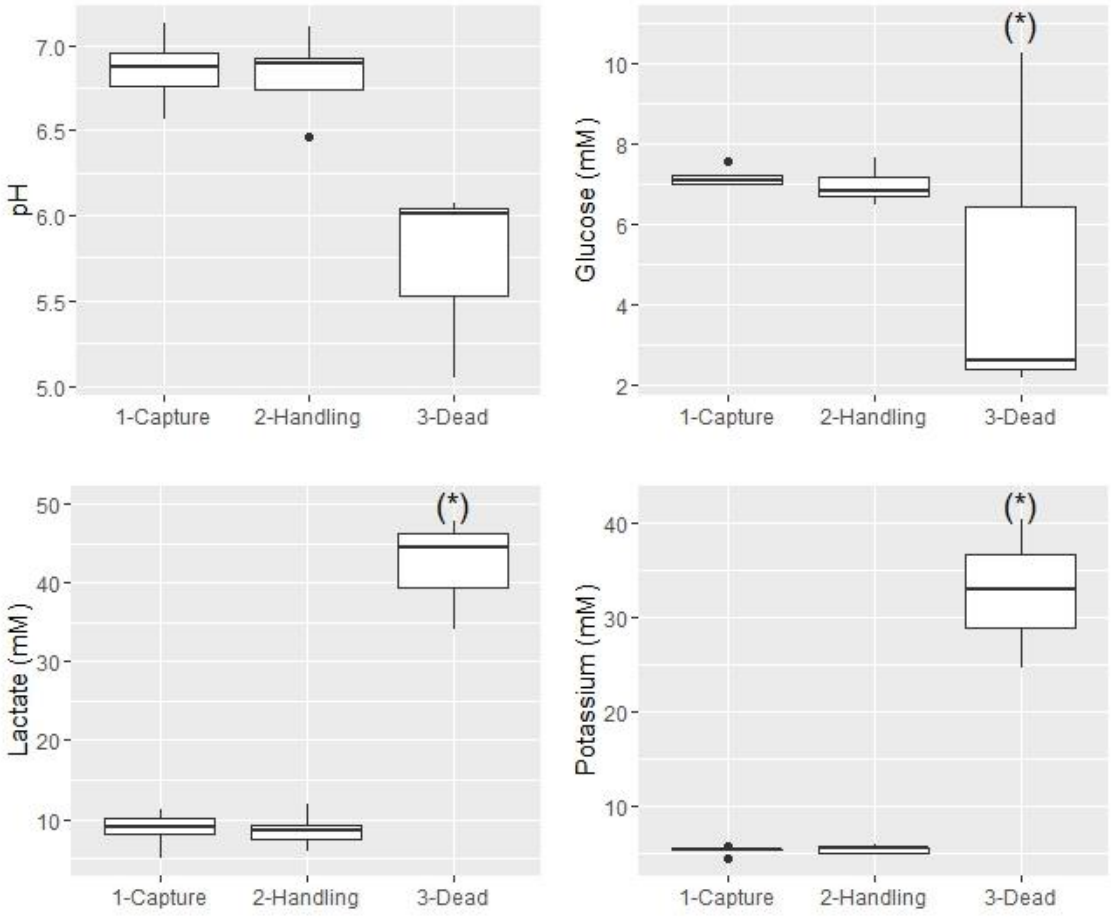



**Figure 2.** Levels of pH, glucose, lactate and potassium in plasma for live (capture and handling) and dead tiger sharks. The symbol (*) represents statistical differences (p=0.05) between live (n=5) and dead sharks (n=3). A Student's t-Test (paired two-samples) was used for differences between capture and handling, and Student's t-Test (unpaired two-sample comparison) was used for differences between live and dead tiger sharks. Each box represents the values grouped, the line represents the mean, the bar represents the standard deviation and the black points represents the outliers.

.

There were no differences for any of the osmolytes or osmolality analyzed between captured and handled samples, except for chloride that showed an increase after handling. Total proteins in plasma was also similar within live sharks (Figure 3 and 4).

Urea plasma concentration was lower in dead animals when compared to live sharks. Sodium, chloride, phosphorus, and osmolality were higher in dead sharks, when compared to live animals. Magnesium, calcium and total proteins values of dead sharks did not show differences when compared to values in live sharks (Figure 3 and 4).

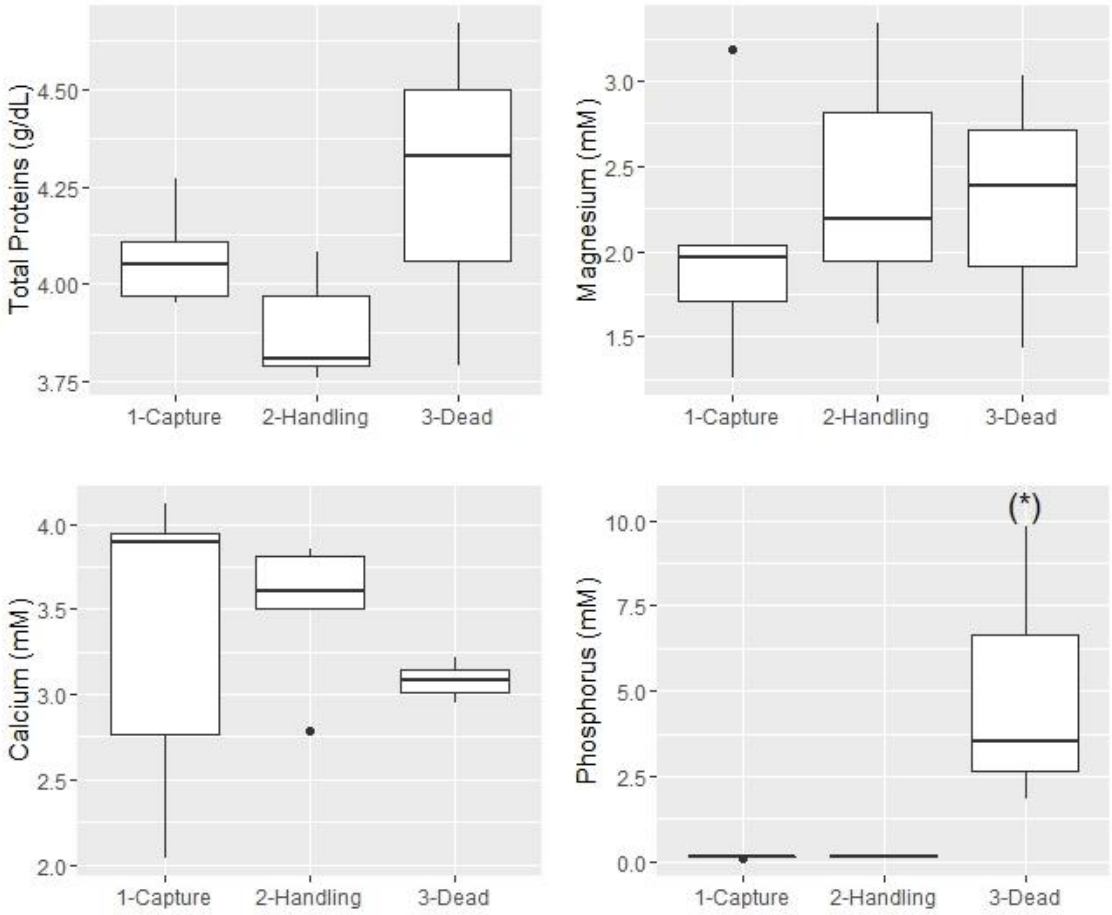



**Figure 3.** Total proteins, magnesium, calcium and phosphorus concentrations in plasma for live (capture and handling) and dead tiger sharks. The symbol (*) represents statistical differences ($p<0.05$) between live (n=5) and dead sharks (n=3). A Student's t-Test (paired two-samples) was used for differences between capture and handling, and Student's t-Test (unpaired two-sample comparison) was used for differences between live and dead tiger sharks. Each box represents the values grouped, the line represents the mean, the bar represents the standard deviation and the black points represents the outliers.

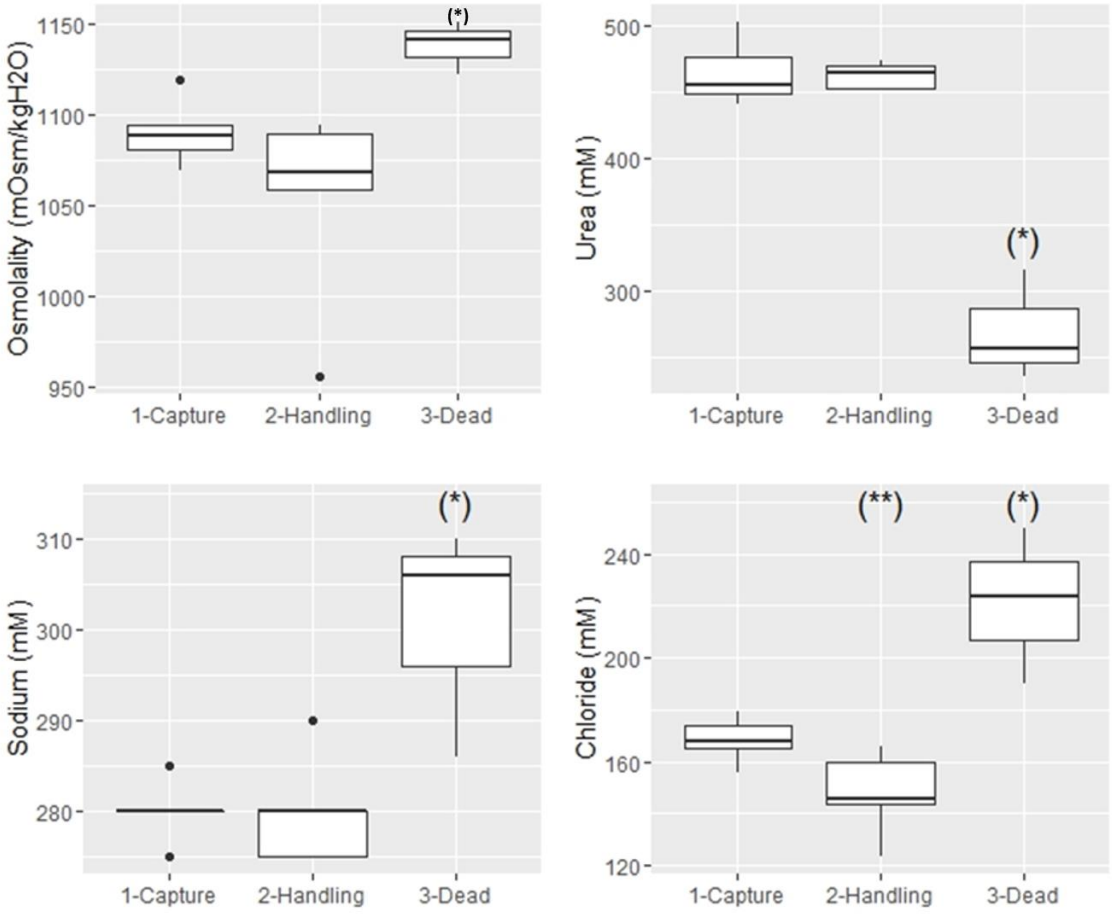



**Figure 4.** Osmolality, urea, sodium and chloride concentrations in plasma for live (capture and handling) and dead tiger sharks. The symbol (*) represents statistical differences ($p<0.05$) between live (n=5) and dead sharks (n=3). The symbol (**) denotes statistical differences between capture (n=5) and handling (n=5) in live sharks. A Student's t-Test (paired two-samples) was used for differences between capture and handling, and Student's t-Test (unpaired two-sample comparison) was used for differences between live and dead tiger sharks. Each box represents the values grouped, the line represents the mean, the bar represents the standard deviation and the black points represents the outliers.

## 4 Discussion

The comparison between live and dead sharks demonstrated that plasma pH, lactate, potassium, phosphorus, osmolality, urea, sodium and chloride showed significant alterations. Thus, it is possible to infer that the parameters cited above are indeed relevant tools for comparative stress studies in tiger sharks. In contrast, total plasma proteins, magnesium and calcium showed no differences between live and dead sharks, and therefore seem not to be adequate to infer severe stress in this species. For glucose, the difference between live and dead sharks was at an individual level, without a clear pattern of modulation

during stress response. As observed in behavioral studies, stressful situations lead to responses at the individual level (Cockrem 2013; Vitousek *et al.* 2014; Brajon *et al.* 2016). Abrupt stressful situations have proven to trigger off even more particular responses (Edwards 1988). Coping strategies are also described as a way to deal with stressful situations, such as salinity changes (Marshall 2003) and captivity (Braithwaite and Ebbesson 2014). So, it is possible that the variable pattern observed in plasma glucose may be related not only to the amount of stress experienced by the individual (at a "personality" level), but also on how the organism in question *copes* with stress (Edwards 1988).

## Problematic of control group in stress studies and *Post-mortem* as a promising field in research

The practice of blood withdrawal, causes discomfort to the animal, potentially generating physiological alterations with respect to ideally unstressed animals (Pickering *et al.* 1982; Barton and Iwama 1991; Foo and Lam 1993). Thus, the need for manipulation makes it impossible to obtain baseline data as controls. The use of anesthetics in sharks during field trips is not recommended, since the time spent out of the water needs to be minimized in order to avoid the deleterious effects of prolonged air exposure and due to the fact that the animals need to be released back to nature in a responsive state (Neiffer and Stamper 2009). However, since data from anesthetized animals can bring new information and improve the understanding of elasmobranch stress response, whenever it is possible, blood withdrawal using anesthesia (*e.g.* in small-sized shark's species that can be maintained in captivity for scientific purposes) should be addressed.

Since it is difficult to establish a baseline reference, the use of negative control through *Post-mortem* analysis might become a viable option since it is possible to verify the extent to which the animal can withstand the physiological stress of capture without succumbing to death. The closer the parameters from live animals are from those levels associated to dead animals (*i.e*., lethal levels of internal parameters), the less healthy the animal are, and death will be then more likely to ensue, caused, at least partly, by the stress of capture. Currently, *Post-mortem* data are rarely used and poorly understood. Living organisms are complex systems composed of highly refined functions (Pozhitkov et al. 2016).

The complexity and proper functioning of an organism are attributes closely related to a series of genetic and physiological regulatory paths, responsible for the maintenance of the homeostasis and link between molecules, cells, organs and systems. Obviously, the traditional approach in the research field is to focus on genetic expression and physiological responses of living organisms. However, there has been growing interest on the use and assays of biological material from recently deceased animals. It is relevant to evaluate how and for how long physiological systems at organs, tissues, and cellular levels keep working after death.

Genetic and physiological responses in dead animals are closely related to the time that it takes to dissipate stored energy and also deceleration of feedback loops (Pozhitkov *et al.* 2016). A recent study showed that expression and upregulation of genes related to the maintenance of homeostasis, such as HSP 70, solute carrier family 26 Anion Exchanger Member 4 (*Slc26a4*), potassium channel voltage gated subfamily H (*Kcnh2*) and urea transporter 2 (*Slc14a2*), occurs for hours to days after death (Pozhitkov *et al.* 2016). This leads us to believe that a better and more complete understanding of *Post-mortem* parameters may bring new and as yet undiscovered genetic and physiological patterns that can help to unveil gaps in studies made with live animals. *Post-mortem* levels may increasingly be considered as reference endpoints for extreme stress in live animals.

## Homeostatic profile of tiger sharks

By definition, homeostasis is the state of internal stability (physical-chemical), within certain limits, even in the face of alterations imposed by the environment (Cannon 1929). Vertebrates are essentially osmoregulators, in that energy is spent in assuring “constant” or at least stable extracellular osmotic concentrations. Stressful situations can cause an imbalance of osmotic/ionic homeostasis. Thus, knowledge of the main osmotically active components in plasma is essential to understand the recovery process or death caused by a stressor. Currently, the majority of stress studies in sharks does not measure the main molecules involved in osmoregulation process so, it is virtually impossible

to determine the homeostatic profile and the role of each osmolyte for the species in question (Table 2).

The analysis conducted here shows that urea has a major role in assuring homeostasis in the species, followed by plasma sodium. Plasma osmolality in elasmobranchs is essentially the sum of NaCl and urea (and TMAO, not assayed here) (Marshall and Grosell 2006; Hammerschlag 2006; Ballantyne and Robinson 2010; Wright and Wood 2015) and these were very different in the dead animals, when compared to both groups of living animals. The result was expected, and entirely consistent with cessation of salt secretion by the rectal gland, causing plasma NaCl levels to increase, and breakdown of urea reabsorption by the kidneys, causing urea titers to decrease in plasma. Other studies of stress of capture performed with *G. cuvier* reported lower values for lactate (around 4 mM - Marshall *et al.* 2012) (3 mM - Gallagher *et al.* 2014), sodium (267 mM), calcium (2.4 mM) and magnesium (1 mM) (Marshall *et al.* 2012), similar values for glucose (6.4 mM) and potassium (5.3 mM) and higher values for chloride (263 mM) (Marshall *et al.* 2012) than those observed in the live animals of the present study. Despite the possible comparisons mentioned above, it is unclear whether the differences in the concentrations observed in other studies actually mean deviation from a homeostatic state, since only few ions and/or osmotic molecules were analyzed.

| Shark species | Urea (mM) | $Na^+$ (mM) | $Cl^-$ (mM) | $Mg^{2+}$ (mM) | $K^+$ (mM) | $Ca^{2+}$ (mM) | $P^{3-}$ (mM) | Osmolality | Glucose (mM) | Lactate (mM) | Total proteins |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Carcharhinus plumbeus*[1] | - | + | + | + | + | + | - | - | + | + | - |
| *Rhizoprionodon terraenovae*[1] | - | + | - | - | + | + | - | - | + | + | - |
| *Carcharhinus limbatus*[1] | - | + | - | - | + | + | - | - | + | + | - |
| *Carcharhinus longimanus*[1] | - | + | - | - | + | + | - | - | + | + | - |
| *Carcharhinus obscurus*[1] | - | + | + | + | + | + | - | - | + | + | - |
| *Isurus oxyrinchus*[1] | - | + | - | - | + | + | - | - | + | + | - |
| *Prionace glauca*[1] | - | + | - | - | + | + | - | - | + | + | - |
| *Carcharhinus falciformis*[1] | - | + | - | - | + | + | - | - | + | + | - |
| *Carcharhinus perezii*[2] | + | + | + | + | + | + | - | - | + | + | - |
| *Prionace glauca*[1] | + | + | + | + | + | + | - | + | + | + | - |
| *Alopias vulpinus*[4] | - | + | + | + | + | + | - | - | + | - | - |
| *Rhizoprionodon terraenovae*[5] | - | - | - | - | - | - | - | + | + | + | - |
| *Carcharhinus limbatus*[6] | - | - | - | - | - | - | - | - | - | + | - |
| *Carcharhinus leucas*[6] | - | - | - | - | - | - | - | - | - | + | - |
| *Sphyrna mokarran*[6] | - | - | - | - | - | - | - | - | - | + | - |
| *Negaprion brevirostris*[6] | - | - | - | - | - | - | - | - | - | + | - |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Squalus acanthias*[7] | + | + | + | + | + | + | - | - | + | + | - |
| *Heterodontus portusjacksoni*[8] | + | - | - | - | + | - | - | - | + | + | - |
| *Mustelus antarcticus*[8] | + | - | - | - | + | - | - | - | + | + | - |

**Table 2**. Urea, sodium, chloride, magnesium, potassium, calcium, phosphorus, osmolality, glucose, lactate and total proteins in several sharks exposed to stress of capture. (+) when data was assessed and (-) when the parameter was not evaluated. Data basen on: (1) Marshall *et al.* 2012; (2) Brooks *et al.* 2012; (3) Wells *et al.* 1986; (4) Heberer *et al.* 2010; (5) Hoffmayer and Parsons 2001; (6) Gallagher *et al.* 2014; (7) Mandelman and Farrington 2007; (8) Frick *et al.* 2010.

Lactate values in dead individuals were exceptionally high in dead sharks when compared to living individuals. Increases in plasma lactate are expected since an increase in the energy demand is observed during capture as a physiological response to fight (partly supported by anaerobic metabolism in white muscle) (Bone 1998; Smith 1992; Pickering and Potinger 1995; Hoffmayer and Parsons 2001). The synthesis and release of lactic acid leads to a decrease in blood pH causing a variety of deleterious effects at the cellular level (Albers 1970; Smith 1992). In the present study, a negative correlation was observed between lactate and pH (Pearson's $R^2$= -0.90, P=0.03). In live animals, the lower plasma lactate concentration was consistent with higher pH values. Dead animals also showed increased lactate, with plasma acidification. Stress and exhaustive exercise from fighting in animals hooked leads to markedly increased lactate and consequent muscle and plasma acidosis, coupled to the inability to ventilate efficiently, resulting in a total loss of acid-basic balance (Wood *et al.* 1983; Bonga 1997; Thomas *et al.* 1999). Plasma lactate and pH are thus closely related parameters that should be evaluated in elasmobranchs stress studies, as also demonstrated by Awruch *et al.* (2001) and Gallagher *et al.* (2014).

Comparing the present data with other studies performed using other species of sharks in good condition vs. moribund/dead animals (Manire et al. 2001; Moyes *et al.* 2006) it was possible to observe that, even within a similar time frame after death (*i.e.,* ~hours), the pattern of loss in homeostasis is not identical (Table 3). The most typical "death markers" are lactate and potassium, which always show a sharp increase. Differences among the species were noted in plasma calcium and magnesium; in tiger sharks these parameters were not different between dead and live animals. The opposite was observed for other species of sharks. The complexity of the regulation of these two cations, coupled with the variability *Post-mortem* suggests that further studies are needed to provide a clearer picture of their loss of homeostasis. Otherwise, NaCl showed differences for tiger sharks but not for other species when comparing dead and live animals. As discussed above, the increase in their plasma levels suggests failure of the rectal gland secretory function. The lack of difference in NaCl levels in dead individuals of other species (see Table 3) with respect to their live counterparts, may be ascribed to a higher sensitivity of these other species to

capture and handling when compared to tiger sharks. Indeed, tiger sharks are known to be very robust and reportedly show high survival rates facing either accidental capture in commercial fisheries (catch and release, Beerkircher *et al.* 2002; Morgan and Burgess 2007), or specific capture for scientific purposes (Gallagher *et al.* 2014).

| | Shark species | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Plasma osmolyte | *S. tiburo** | | *C. limbatus** | | *C. leucas** | | *P. glauca#* | | *G. cuvier#* | | |
| | Live (4) | Dead (12) | Live (9) | Dead (8) | Live (11) | Dead (5) | Live | Dead | Capture (5) | Handling (5) | Dead (3) |
| Osmolality | - | - | - | - | - | - | - | - | 1090±8.3 | 1053±25.1 | 1138±8.5 |
| Urea | - | - | - | - | - | - | 357±4 (11) | 352±11 (8) | 464±11 | 462±4.4 | 269±23.8 |
| Sodium | 312 (306-317) | 301 (286–306) | 321 (313–329) | 328 (321–333) | 288 (285–294) | 283 (278–291) | 263±1 (11) | 264±4 (7) | 280±1.58 | 280±2.73 | 300±7.4 |
| Chloride | 207 (206–209) | 210 (205–213) | 208 (207–212) | 212 (210–214) | 202 (201–204) | 199 (198–200) | 240±4 (11) | 236±5 (7) | 168±4.02 | 147±18.7 | 221±17.4 |
| Potassium | 6.4 (6.0–7.1) | 16.1 (11.4–17.5) | 4 (3.7–4.3) | 7.3 (7.0–9.7) | 6.3 (5.7–6.7) | 10.1 (9.4–12.9) | 5.12±0.44 (11) | 7.01±0.66 (9) | 5.26±0.2 | 5.32±0.18 | 33.53±4.5 |
| Calcium | 16.5 (15.9–17.2) | 16.8 (15.8–17.5) | 17.1 (16.3–17.7) | 19.9 (18.4–21.7) | 17.3 (16.0–17.6) | 18.6 (17.2–19.0) | 3.13±0.11 (11) | 3.70±0.14 (9) | 3.3±0.4 | 3.48±0.1 | 3.06±0.07 |
| Magnesium | - | - | - | - | - | - | 0.98±0.05 (11) | 1.57±0.08 (9) | 2±2.95 | 2.35±2.94 | 2.26±0.4 |
| Lactate | 3.9 (2.2–5.3) | 12 (12.0–12.0) | 4.7 (4.1–6.4) | 12 (11.2–12.0) | 6.3 (4.5–11.8) | 12 (12.0–12.0) | 5.80±2.96 (11) | 27.7±24.07 (9) | 8.72±1.04 | 8.62±1.01 | 42.13±4.1 |
| Glucose | 183 (175-192) | 125 (115–141) | 62 (48.5–67.3) | 44 (31.5–60.0) | 54.5 (41.0–64.0) | 22 (11.5–32.3) | 4.75±0.40 (11) | 4.16±0.69 (9) | 7.16±1.06 | 6.98±2.05 | 5.01±2.6 |
| T. Proteins | 3.2 (3.0–3.3) | 3.9 (3.7–4.3) | 2.2 (2.2–2.6) | 2.4 (2.1–2.7) | 2.9 (2.8–2.9) | 2.6 (2.3–2.8) | - | - | 4.08±0.05 | 3.9±0.06 | 4.26±0.25 |
| Phosphorus | - | - | - | - | - | - | - | - | 0.14±0.01 | 0.15±0.01 | 5.05±2.4 |

**Table 3**. References used: *S. tiburo*, *C. limbatus* and *C. leucas* (Manire et al. 2001); *P. glauca* (Moyes et al. 2006); *G. cuvier*, current study (same data depicted in Figures 1, 2, and 3). *Values are median ($25^{th}$-$75^{th}$ quartiles), and # mean ± SEM (n).

## Glucose: the need for caution when employed as stress marker

In vertebrates, as a primary response to stress, catecholamine and corticosteroids are released into the bloodstream (de Roos and de Roos 1978). As a result, the glycogen produced and stocked in the liver is mobilized to face the energy demands of the animal. However, in sharks, the major source of oxidative fuel are ketone bodies and amino acids (Speers-Roesch and Treberg

2010; Ballantyne 2015), and not glucose as observed in mammals and birds (Boosntra 2004; Romero and Butler 2007; Wright *et al.* 2011). Nevertheless, hyperglycemia is commonly used in studies as an indicator of stress in sharks (Table 2). Characterization of urea transporters in sharks demonstrates that during hyperglycemia glucose receptors located in the rectal gland are activated (Walsh *et al.* 2006; Deck *et al.* 2016). Thus, high plasma glucose concentration in sharks is probably responsible to maintain the secretory mechanism (NaCl), using glucose as a metabolic fuel in an attempt to restore the homeostatic balance and not for aerobic metabolism. Also, a recent study hypothesized the use of plasma glucose by the shark's brain as crucial for the metabolic coupling between glia and neurons (Balmaceda-Aguilera *et al.* 2012).

Unlike commonly reported, the present study showed *post-mortem* hypoglycemia in two of three analyzed dead sharks (possibly related to the exhaustion from capture). Studies performed with bonnethead (*S. tiburo*), blacktip (*C. limbatus*) and bull (*C. leucas*) sharks showed hypoglycemic response in moribund/death sharks (Manire et al. 2001), corroborating at least partially to the findings of the present study. Post-release survival analysis performed in blue sharks (*P. glauca*) showed no difference between plasma glucose concentrations in moribund and live sharks (Moyes *et al.* 2006), proposing that the hyperglycemic profile commonly cited as a reliable indicator may not be directly related to lethality. Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*) exposed to different stressors (hypoxia exposure and fasting) also did not exhibited hyperglycemia as response (Hoffmayer *et al.* 2015). The high glucose concentration often observed in stress studies may be related to a series of factors not related to capture or handling, such as genetic predisposition, circadian rhythms (Gutierrez *et al.* 1984), feeding responses or as an immediate (short-term) reaction to a non-resting state. It is hypothesized that sharks are at an earlier stage in the glucose regulation evolutionary path (Ballantyne 2015). The "poor" regulation of glucose is probably related not only to the inability of kidneys to proper regulate the molecule, but also for its reduced role in elasmobranch's energy intake (Ballantyne 2015). Such is the lack of pattern for glucose in tiger sharks that the same concentrations observed in the present study (less than 30 minutes hooked) were observed in tiger sharks captured by

longline hooked for 2-12 hours (Marshall *et al.* 2012). Also, stressors such as severe hypoxia (Routley *et al.* 2002; Speers-Roesch and Treberg 2010; Speers-Roesch *et al.* 2012) and long term fasting (de Rooset al. 1985) did not cause changes in glucose levels, suggesting the weak association between this compound and the main stress response mechanisms. Finally, Mommsen et al. (1999) discuss how the role of glucose is often overrated in its importance in fish. Thus, the use of glucose to assess stress should be treated with caution, since there are many variables that may affect its concentration.

Urea and phosphorus as stress parameters

Despite the proven importance of urea as the most significant molecule responsible for extracellular osmotic stability in the group (Ballantyne and Robinson 2010; Ballantyne 2016; Treberg and Speers-Roesch 2016), few studies have analyzed plasma alterations of urea upon the stress of capture and handling in sharks (Moyes *et al.* 2006; Mandelman and Farrigton 2007; Frick *et al.* 2010; Brooks et al. 2012) (Table 2). Such is the importance of this compound for sharks that about 14% of the oxygen consumption is reserved for urea synthesis (Kirschner 1993) and the ability to decrease urea in plasma seem to be determinant for the evolutionary occupation of freshwater environments (Ballantyne and Robinson 2010). A reduction of 57% in urea concentration was observed between live and dead sharks, clearly demonstrating an inability to maintain the urea balance and as consequence concentrations compatible to life. The decrease observed was probably caused by membrane rupture, loss of retention capacity and reduced renal reabsorption of urea via the countercurrent system, as suggested above, with the cessation of blood pumping by the heart (Ballantyne and Robinson 2010). These results suggest that the analysis of plasma urea might be useful to assess homeostatic changes caused by stress (death) in sharks and should not, therefore, be neglected.

Plasma phosphorus, which has been even more overlooked (Table 2), showing values five times higher in dead animals when compared to concentrations found in live animals. It is a compound involved in cell growth/differentiation, cell membrane formation and phospholipids production. Phosphorus imbalance can also lead to changes in calcium regulation, since both

are closely related (Andrigueto *et al.* 1990). Hyperphosphatemia is detrimental, since it leads to phosphate crystals formation which block arteries, causing heart failure (Neves *et al.* 2004). The higher concentrations of phosphorus in plasma observed in the present study may be related to the disintegration of cellular membranes, renal failure and gill collapse (Albers 1970). Although rarely used, plasma phosphorus also seems to be an effective and unexpansive tool to assess stress level in this species.

## 5 Conclusion

By comparing surviving and dead tiger sharks, it was possible to conclude that: (1) *Post-mortem* data may be used as endpoint references in studies of stress in *G. cuvier*; (2) the sharks caught, sampled and released alive in the present study did not show loss of plasma osmotic/metabolic homeostatic balance when compared to *Post-mortem* data, indicating that the procedures did not generate a significant stress response; (3) the use of glucose as a tool for stress analysis should be treated with caution since: (a) hyperglycemia observed after capture may be related to other factors and (b) elasmobranchs do not use glucose as metabolic fuel. Finally, (4) urea and phosphorus concentrations, often neglected, are in fact very relevant for osmotic and ionic balance and, for that reason, should be analyzed in stress studies.

## Literature cited

Albers, C. (1970). Acid-base balance. In: Fish Physiology. Hoar W, Randall DJ (eds). Vol. IV. Academic Press Inc., United States pp.173-205.

Anderson, W. G. (2012). The endocrinology of 1α-hydroxycorticosterone in elasmobranch fish: A review. Comparative Biochemistry and Physiology (A), 162(2),73-80.

Andrigueto, J. M., Peryl, L., Minardi, I. (1990). Nutrição animal: bases e fundamentos da nutrição animal. Vol. I. Nobel 1, Brazil 396pp.

Armour, K. J., O'Toole, L. B., Hazon, N. (1993). The effect of dietary protein restriction on the secretory dynamics of 1α-hydroxycorticosterone and urea in the dogfish, *Scyliorhinus canicula*: a possible role for 1α-hydroxycorticosterone in sodium retention. Journal of Endocrinology, 138(2), 275-282.

Awruch, C. A., Simpfendorfer, C., Pankhurst, N. W. (2011). Evaluation and use of a portable field kit for measuring whole-blood lactate in sharks. Marine and Freshwater Research, 62, 694-699.

Ballantyne, J. S., Robinson, J. W. (2010). Freshwater elasmobranchs: a review of their physiology and biochemistry. Journal of Comparative Physiology (B), 180(4), 475-493.

Ballantyne, J. S. (2015). Jaws II: Metabolism of Elasmobranchs. In: Physiology of Elasmobranch Fishes: Internal Processes. Shadwick RE, Farrell AP, Brauner CJ (eds). 1st edition. Academic Press, United States, pp. 395-456.

Ballantyne, J. S. (2016). Some of the most interesting things we know, and don't know, about the biochemistry and physiology of elasmobranch fishes (sharks, skates and rays). Comparative Biochemistry and Physiology (B), 199, 21-28.

Balmaceda-Aguilera, C., Cortes-Campos, C., Cifuentes, M., Peruzzo, B., Mack, L., Tapia, J. C., et al. (2012). Glucose transporter 1 and monocarboxylate transporters 1, 2, and 4 localization within the glial cells of shark blood-brain-barriers. PloS one 7(2):e32409.

Barton, B. A., Iwama, G. K. (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Review of Fish Diseases, 1, 3-26.

Beerkircher, L. R., Cortes, E., Shivji, M. (2002). Characteristics of shark's bycatch observed on pelagic loglines off Southeastern United States, 1992-2000. Marine Fisheries Review, 64(4), 40-49.

Bone, Q. (1988). Muscles and locomotion. In: Physiology of elasmobranch fishes. Shuttleworth TJ (editor). Springer-Verlag, Berlin, pp.99–141.

Bonga, S. W. (1997). The stress response in fish. Physiological Reviews, 77(3), 591-625.

Boonstra, R. (2004). Coping with changing northern environments: the role of the stress axis in birds and mammals. Integrative and Comparative Biology, 44(2), 95-108.

Braithwaite, V. A., Ebbesson, L. O. E. (2014). Pain and stress responses in farmed fish. Revue Scientifique et Technique, 33(1), 245-253.

Brajon, S., Laforest, J. P., Schmitt, O., Devillers, N. (2016). A preliminary study of the effects of individual response to challenge tests and stress induced by humans on learning performance of weaned piglets (*Sus scrofa*). Behavioral Process, 129, 27-36.

Brooks, E. J., Sloman, K. A., Liss, S., Hassan-Hassanein, L., Danylchuk, A. J., Cooke, S. J., Mandelman, J. W., Skomal, G. B., Sims, D. W., Suski, C. D. (2011). The stress physiology of extended duration tonic immobility in the juvenile lemon shark, *Negaprion brevirostris* (Poey 1868). Journal of Experimental Marine Biology and Ecology, 409, 351–360.

Brooks, E. J., Mandelman, J. W., Sloman, K. A., Liss, S., Danylchuk, A. J., Cooke, S. J., Skomal, G. B., Philipp, D. P., Sims, D. W., Suski, C. D. (2012). The physiological response of the Caribbean reef shark (*Carcharhinus perezi*) to longline capture. Comparative Biochemistry and Physiology (A), 162, 94–100.

Cannon, W. B. (1929). Organization for physiological homeostasis. Physiological Reviews, 400, 399-431.

Cliff, G., Thurman, G. D. (1984). Pathological and physiological effects of stress during capture and transport in the juvenile dusky shark, *Carcharhinus obscurus*. Comparative Biochemistry and Physiology (A), 78,167–173.

Cockrem, J. F. (2013). Individual variation in glucocorticoid stress responses in animals. General and Comparative Endocrinology, 181, 45-58.

Crowder, L. B., Murawski, S. A. (1998). Fisheries bycatch: implications for management. Fisheries, 23(6), 8-17.

Davies, R. W. D., Cripps, S. J., Nickson, A., Porter, G. (2009). Defining and estimating global marine fisheries bycatch. Marine Policy, 33(4), 661-672.

deRoos, R., deRoos, C. C. (1978). Elevation of plasma glucose levels by catecholamines in elasmobranch fish. General and Comparative Endocrinology, 34, 447–452.

deRoos, R., deRoos, C. C., Werner, C. S., Werner, H. (1985). Plasma levels of glucose, alanine, lactate, and β-hydroxybutyrate in the unfed spiny dogfish (*Squalus acanthias*) after surgery and following mammalian insulin infusion. General and Comparative Endocrinology, 58, 28–43.

Deck, C. A., LeMoine, C. M. R., Walsh, P. J. (2016). Phylogenetic analysis and tissue distribution of elasmobranch glucose transporters and their response to feeding. Biology Open, 0, 1-6.

Dulvy, N. K., Baum, J. K., Clarke, S., Compagno, L. J., Cortes, E., Domingo, A., et al. (2008) You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. Aquatic Conservation: Marine and Freshwater Ecosystems, 18(5), 459-482.

Edwards, J. R. (1988). The determinants and consequences of coping with stress. In: Causes, Coping Consequences of Stress at Work. Coopre CL and Payn R (eds). Chichester, John Wiley and Sons, pp.233-266.

FAO (2011). International guidelines on bycatch management and reduction of discards. 73 pp.

Frick, L. H., Reina, R. D., Walker, T. I. (2010). Stress related physiological changes and post-release survival of Port Jackson sharks (*Heterodontus portusjacksoni*) and gummy sharks (*Mustelus antarcticus*) following gill-net and longline capture in captivity. Journal of Experimental Marine Biology and Ecology, 385, 29–37.

Foo, J. T. W., Lam, T. J. (1993). Serum cortisol response to handling stress and the effect of cortisol implantation on testosterone level in the tilapia *Oreocrhomis mossambicus*, Aquaculture, 11(5), 145-158.

Gallagher, A., Serafy, J., Cooke, J. S., Hammerschlag, N. (2014). Physiological stress response, reflex impairment, and survival of five sympatric shark species following experimental capture and release. Marine Ecology Progress Series, 496, 207–218.

Gutierrez, J., Carrillo, M., Zanuy, S., Planas, J. (1984). Daily rhythms of insulin and glucose levels in the plasma of sea bass *Dicentrarchus labrax* after experimental feeding. General and Comparative Endocrinology, 55(3), 393-7.

Hammerschlag, N. (2006). Osmoregulation in elasmobranchs: a review for fish biologists, behaviourists and ecologists. Marine and Freshwater Behavior and Physiology, 39(3), 209-228.

Hazin, F. H. V., Afonso, A. S. (2013). A green strategy for shark attack mitigation off Recife, Brazil. Animal Conservation ,17(4), 287–296.

Hazon, N., Henderson, I.W. (1984). Secretory dynamics of 1α-hydroxycorticosterone in the elasmobranch fish, *Scyliorhinus canicula*. Journal of Endocrinology, 103(2), 205-211.

Heberer, C., Aalbers, S. A., Bernal, D., Kohin, S., DiFiore, B., Sepulveda, C. A. (2010). Insights into catch-and-release survivorship and stress-induced blood biochemistry of common thresher sharks (*Alopias vulpinus*) captured in the southern California recreational fishery. Fisheries Research, 106, 495–500.

Hoffmayer, E.R., Parsons, G.R. (2001). The physiological response to capture and handling stress in the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*. Fish Physiology and Biochemistry, 25, 277–285.

Hoffmayer, E. R., Hendon, J. M., Parsons, G. R. (2012). Seasonal modulation in the secondary stress response of a carcharhinid shark, *Rhizoprionodon terraenovae*. Comparative Biochemistry and Physiology (A), 162(2), 81-87.

Hoffmayer, E. R., Hendon, J. M., Parsons, G. R., Driggers, W. B., Campbell, M. D. (2015). A comparison of single and multiple stressor protocols to assess acute stress in a coastal shark species, *Rhizoprionodon terraenovae*. Fish Physiology and Biochemistry, 41(5), 1253-60.

Hyatt, M. W., Anderson, P. A., O'Donnell, P. M. (2016). Behavioral release condition score of bull and bonnethead sharks as a coarse indicator of stress. Journal of Coast Research, 32(6), 1464-1472.

Idler, D. R., Truscott, B. (1966). 1α-hydroxycorticosterone from cartilaginous fish: a new adrenal steroid in blood. Journal of the Fisheries Research Board of Canada, 23(4), 615-619.

Kirschner, L. B. (1993). The energetics of osmotic regulation in ureotelic and hypoosmotic fishes. Journal of Experimental Zoology, 267, 19–26.

Mandelman, J. W., Farrington, M. A. (2007). The physiological status and mortality associated with otter-trawl capture, transport, and captivity of an exploited elasmobranch, *Squalus acanthias*. ICES Journal of Marine Science, 64, 122–130.

Mandelman, J. W., Skomal, G. B. (2009). Differential sensitivity to capture stress assessed by blood acid–base status in five carcharhinid sharks. Journal of Comparative Physiology (B), 179, 267–277.

Manire, C., Hueter, R., Hull, E., Spieler, R. (2001). Serological changes associated with gill-net capture and restraint in three species of sharks. Transactions of the American Fisheries Society, 130, 1038–1048.

Manire, C. A., Rasmussen, L. E. L., Maruska, K. P., Tricas, T. C. (2007). Sex, seasonal, and stress-related variations in elasmobranch corticosterone concentrations. Comparative Biochemistry and Physiology (A), 148(4), 926-935.

Marshall, W. S. (2003). Rapid regulation of NaCl secretion by estuarine teleost fish: coping strategies for short-duration freshwater exposures. Biochimica et Biophysica Acta: Reviews on Biomambranes, 1618(2), 95-105.

Marshall, W. S., Grosell, M. (2006). Ion transport, osmoregulation, and acid-base balance. In: The physiology of fishes. Evans DH, Claiborne JB, Currie S (eds). CRC Press, Boca Raton, USA, pp.177-230

Marshall, H., Field, L., Afiadata, A., Sepulveda, C., Skomal, G., Bernal, D. (2012). Hematological indicators of stress in longline-captured sharks. Comparative Biochemistry and Physiology (A), 162, 121–129.

Mommsen, T. P., Vijayan, M. M., Moon, T.W. (1999). Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology Fisheries, 9, 211–268.

Morgan, A., Burgess, G. H. (2007). At-vessel fishing mortality for six species of sharks caught in the northwest Atlantic and Gulf of Mexico. Proceedings of the 59th Annual Conference of the Gulf and Caribbean Fisheries Institute, 19, pp 123–130

Moyes, C. D., Fragoso, N., Musyl, M. K., Brill, R. W. (2006). Predicting post release survival in large pelagic fish. Transactions of the American Fisheries Society, 135, 1389–1397.

Neiffer, D. L., & Stamper, M. A. (2009). Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. ILAR journal, 50(4), 343-360.

Neves, K. R., Graciolli, F. G., Dos Reis, L. M., Pasqualucci, C. A., Moyses, R. M., Jorgetti, V. (2004). Adverse effects of hyperphosphatemia on myocardial hypertrophy, renal function, and bone in rats with renal failure. Kidney International, 66(6), 2237-2244.

Pickering, A. D., Pottinger, T. G., Christie, P. (1982). Recovery of the brown trout, *Salmo trutta L*., from acute handling stress: a time-course study. Journal of Fish Biology, 20(2), 229-244.

Pikering, A. D., Pottinger, T. G. (1995). Biochemical effects of stress. In: Environmental and ecological biochemistry. Hochachka PW, Mommsen TP (eds.). Amsterdam: Elsevier, pp 349-379

Rasmussen, L. E. L., Crow, G. L. (1993). Serum corticosterone concentrations in immature captive whitetip reef sharks, *Triaenodon obesus*. Journal of Experimental Zoology, 267(3), 283-287.

Rasmussen, L. E. L., Gruber, S. H. (1993). Serum concentrations of reproductively-related circulating steroid hormones in the free-ranging lemon shark, *Negaprion brevirostris*. In: The reproduction and development of sharks, skates, rays and ratfishes, Demski, LS, Wourms, JP (eds). Springer Netherlands, pp167-174.

Romero, M. L., Butler, L. K. (2007). Endocrinology of stress. Int J Comp Psychol, 20(2), 89-95.

Routley, M. H., Nilsson, G. E., Renshaw, G. M. C. (2002). Exposure to hypoxia primes the respiratory and metabolic responses of the epaulette shark to progressive hypoxia. Comparative Biochemistry and Physiology (A), 131, 313–321.

Skomal, G. B. (2006). The Physiological Effects of Capture Stress on Post-Release Survivorship of Sharks, Tunas and Marlins. Boston, Massachusetts: Boston University, Ph.D. dissertation, 294pp.

Skomal, G. B. (2007). Evaluating the physiological and physical consequences of capture on post-release survivorship in large pelagic fishes. Fisheries Management Ecology, 14, 81–89.

Skomal, G. B., Bernal, D. (2010). Physiological responses to stress in sharks. In: Sharks and Their Relatives II: Biodiversity, Adaptive Physiology, and Conservation. Carrier J, Musick J, Heithaus, M (eds). CRC Press, Boca Raton, USA, pp 459–490.

Skomal, G. B., Mandelman, J. W. (2012). The physiological response to anthropogenic stressors in marine elasmobranch fishes: a review with a focus on the secondary response. Comparative Biochemestry and Physiology (A), 162, 146–155.

Skomal, G. B., Lobel, P. S., Marshall, G. (2007). The use of animal-borne imaging to assess post release behavior as it relates to capture stress in grey reef sharks, *Carcharhinus amblyrhynchos*. Marine Technology Society Journal, 41, 44–48.

Smith, M. F. L. (1992). Capture and transportation of elasmobranchs, with emphasis on the grey nurse shark, *Carcharias taurus*. Australian Journal of Marine and Freshwater Research, 43, 325–343.

Speers-Roesch, B., Treberg, J. R. (2010). The unusual energy metabolism of elasmobranch fishes. Comparative Biochemistry and Physiology (A), 155, 417-434.

Speers-Roesch, B., Richards, J. G., Brauner, C. J., Farrell, A. P., Hickey, A. J. R., Wang, Y. S., Renshaw, G. M. C. (2012). Hypoxia tolerance in elasmobranchs I. Critical oxygen tension as a measure of blood oxygen transport during hypoxia exposure. Journal of Experimental Biology, 215, 93-102.

Thomas, P. M., Pankhurst, N. W., Bremner, H. A. (1999). The effect of stress and exercise on post-mortem biochemistry of Atlantic salmon and rainbow trout. Journal of Fish Biology, 54(6), 1177-1196.

Treberg, J. R., Speers-Roesch, B. (2016). Does the physiology of chondrichthyan fishes constrain their distribution in the deep-sea? Journal of Experimental Biology, 219, 615-625.

Vitousek, M. N., Jenkins, B. R., Safran, R. J. (2014). Stress and success: Individual differences in the glucocorticoid stress response predict behavior and reproductive success under high predation risk. Hormones and Behavior, 66(5), 812-819.

Walsh, P. J., Kajimura, M., Mommsen, T. P., Wood, C. M. (2006). Metabolic organization and effects of feeding on enzyme activities of the dogfish shark (*Squalus acanthias*) rectal gland. Journal of Experimental Biology, 209, 2929–2938.

Wells, R. M. G., McIntyre, R. H., Morgan, A. K., Davie, P. S. (1986). Physiological stress responses in big gamefish after capture: observations on plasma chemistry and blood factors. Comparative Biochemistry and Physiology (A), 84, 565–571.

Whitney, N. M., White, C. F., Gleiss, A. C., Schwieterman, G. D., Anderson, P., Hueter, R. E., Skomal, G. B. (2016). A novel method for determining post-release mortality, behavior, and recovery period using acceleration data loggers. Fisheries Research, 183, 210-221.

Wingfield, J. C., Romero, L. M. (2001). Adrenocortical responses to stress and their modulation in free-living vertebrates. Comparative Physiology.

Wood, C. M., Turner, J. D., Graham, M. S. (1983). Why do fish die after severe exercise? Journal of Fish Biology, 22, 189–201.

Wright, A. J., Deak, T., Parsons, E. C. M. (2011). Size matters: management of stress responses and chronic stress in beaked whales and other marine mammals may require larger exclusion zones. Marine Pollution Bulletin, 63(1), 5-9.

Wright, P. A., Wood, C. M. (2015). Regulation of ions, acid-base, and nitrogenous wastes in Elasmobranchs. In: Physiology of Elasmobranch Fishes: Internal Processes Farrel AP, Brauner CJ (eds). Academic Press, London 279.

# CAPÍTULO 3

**Heat signature and lactate profile in the nurse shark *Ginglymostoma cirratum*: implications on fight response during capture and air exposure**

**Abstract**

The nurse shark (*Ginglymostoma cirratum*) is a benthic species found in tropical and subtropical waters of the Atlantic Ocean. The Western Atlantic subpopulation is listed as Near Threatened, based on its Vulnerable status off South America. Due to its sedentary habits and primary breathing mechanism (buccal pumping), the species exhibit the lowest metabolic rates among sharks, being extremely resistant to commercial capture and air exposure. The present study aimed to determine thermal surface body profiles of nurse sharks following experimental capture. Using thermal imaging, we documented a consistent heat signature at nurse shark's flanks independent of the environmental conditions at the day of capture. Allied to thermal data, we provide plasma lactate concentrations of each shark experimentally exposed. Our thermal results points to an enhanced muscular response detected by dissipation of heat in the region of greater muscular insertion. The low lactate levels assessed here show a link between the enhanced aerobic activity by the red musculature and a delayed anaerobic response, feature that may help to characterize the robustness of the species facing capture. These data are imperative for comparative purposes, since sedentary species can bring insights on stress response that will help in the understanding of fishing impacts and the improvement of management protocols based on catch and release protocols. Finally, our data brings evidence that thermal imagery can be in fact an efficient tool for diagnosis and assessment of chances in body temperature, allowing a better understand of the physiological paths activated during stressful situations and as consequence shark's conservation in a fishing perspective.

## 1 Introduction

Studies on physiological and behavioral responses to the stress of capture of sharks have increased our understanding of the sensitivity of some species in commercial, recreational and scientific. The ability to cope with the homeostatic disruption caused by stress is directly related to the individual capacity to respond properly by activating a series of physiological mechanisms, such as a rapid increase in the metabolic scope (Skomal and Bernard 2010). Because stress responses are closely related to energy expenditure, and to the ability to quickly recovery from deleterious effects of capture (Cliff and Thurman 1978; Brill *et al.*

2008; Gallagher *et al.* 2014), the assessment of metabolic rates, muscle energetics and secondary stress response is imperative for determining the physiological traits that increase resistance to fishing. In addition, physiological and morphological adaptations of sharks associated with ecology and lifestyle may influence the response when caught (e.g. mode of gill ventilation: buccal-pump and ram ventilator) (Guida *et al.* 2016; Ellis *et al.* 2016).

The physiology and ecology of body temperature is a crucial component in the assessment of thermoregulation; and relates to key variables, such as energy flow, metabolic rates, activity patterns, and food consumption (Kuraoka and Nakamura 2011; Terrien *et al.* 2011; Nowack *et al.* 2013; Thompson *et al.* 2015). New insights in the physiology of thermoregulation became possible with the use of infrared technology (IRT). This non-invasive technique allows evaluating body surface temperature, adjustments of peripheral blood flow, thermogenesis, evaporative cooling and respiratory physiology (Cilulko *et al.* 2013; Usamentiaga *et al.* 2014; Tattersall *et al.* 2016). IRT also allowed the assessment of bouts of endothermy previously unknown for ectothermic animals (Tattersall *et al.* 2016). However, studies using this technology are scarce for aquatic animals, and so far, have focused only on marine mammals (Cuyler *et al.* 1992; Mauck *et al.* 2003; Pabst *et al.* 2002; McCafferty *et al.* 2007).

In parallel to the previous discoveries, IRT has been widely useful to study thermoregulatory patterns in aquatic fish, under both natural and applied contexts. More specifically, this tool has promoted understanding impacts of exposure to air from previously unexplored points of view, particularly the direct exposure to air temperature and solar radiation. This application of IRT permits not only a better comprehension of warm-shock associated to the transition from water to air, but can also raise important points regarding the effects of the reciprocal cold-shock to which animals are exposed when returned to their natural environment. Under the latter perspective, we propose the nurse shark *Ginglymostoma cirratum* as a good model to investigate impacts of fishing practices from the perspective of induced thermal shocks, mainly due to its sendentary habits and high survival rates facing capture and air exposure. We use IRT combined with plasma lactate, a traditional stress marker for sharks (Hoffmayer and Parsons 2001; Brill *et al.* 2008; Frick *et al.* 2009). This metabolite

is linked to the anaerobic capacity of the animal being its circulating levels species-specific (Gallagher *et al.* 2014; Heard *et al.* 2014).

The nurse shark is an inshore bottom-dwelling species, found in tropical and subtropical waters both in continental and insular shelves of the Atlantic Ocean (Compagno 2001). This shark often thrives at depths lower than one meter, but may occur down to 75 m (Compagno 2001). The species exhibits the lowest metabolism rates among sharks (Whitney *et al.* 2016) that parallels about the lowest mortality rates facing capture among sharks (Afonso *et al.* 2011), being able to survive up to eight hours of air exposure (*personal communication,* Hammerschlag 2016). Questions related to post-capture resilience can be investigated in the nurse shark due to its sedentary habits and primary breathing mechanism (buccal pumping), two combined characteristics that make this species particularly interesting for studies of metabolism and thermal dynamics.

## 2 Material and methods

### 2.1 Shark Sampling

Eighteen *G. cirratum* (7 females and 11 males) were captured using a standardized circle-hook drumline system as part of ongoing research surveys made by the Shark Research and Conservation Laboratory (University of Miami) as described in Gallagher et al. (2014). Briefly, gear consisted of a submerged weight with two attachment points: 1) a line running to the surface with buoy floats and 2) a swivel connecting a 23 m monofilament gangion line (~400 kg test) that terminates with a baited 16/0 5°-offset circle hooks (Figure 1).

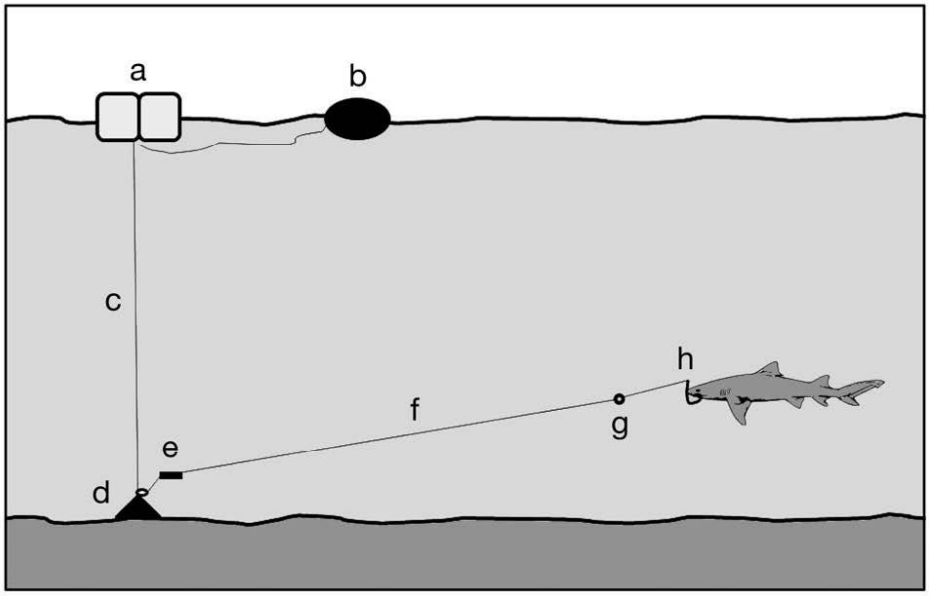



**Figure 1.** Drumline fishing apparatus: (a) bullet floats; (b) large floating poly-ball; (c) rope attaching float to submerged weight; (d) ~18kg cement weight; (e) hook timer; (f) main line of ~410 kg test monofilament; (g) 4 m double-stranded leader of ~410 kg test monofilament; (h) 16/0 5°-offset circle hook. The distance between (e) and (h) is 23 m, and the length between (a) and (d) ranged from 30 to 60m. (Gallagher *et al.* 2014).

The proximal end of the monofilament line was connected to the weight via a hook timer (Lindgren Pitman HT600) that consists of a magnetic release timer to display the time each animal was on the line. Upon capture, animals were removed from the water and placed on a boat-attached platform for sampling. Total length (TL-cm), sex, water and air temperature and hooking duration (*i.e.* fight time) were recorded for each animal.

Experimental procedures and animal husbandry were approved by the University of Miami Institutional Animal Care and Use Committee (Protocol 15-238) and research permits from Florida Fish and Wildlife Conservation Commission, Biscayne National Park and National Marine Fisheries Service.

## 2.2 Infrared Thermography

Thermal images were taken using a FLIR camera (model T420-62101 Wilsonville, Oregon, EUA) during the first minute after landing until the last moment before release. Emissivity coefficient was set at 0.98 – 0.99 units of energy according to the value established for skin in animal research. Each thermal image was analyzed using the FLIR software tools (FLIR® Systems, Inc. version 5.3.15268.1001, 2016). Mean temperatures of the dorsal and lateral

(flanks) axis of the sharks were calculated from 12 points of temperature of each region derived from the thermal images taken. The images were taken at landing until release (~15 minutes) of the animals to assess the thermal profile during the whole time of air exposure.

### 2.3 Blood Sampling and Lactate Assays

Whole blood (5-7 ml) was drawn from each individual via caudal venipuncture right before release. About 4 ml was separated into two vials and spun via centrifugation (13,000 g for 5 minutes) for plasma extraction. Vials containing plasma were stored on ice until frozen at -20ºC. Lactate values were measured on plasma by adding 10 µl of plasma to a lactate meter (Lactate Pro LT-1710 portable lactate analyzer; Arkray Inc., Kyoto, Japan; see Cooke et al., 2008 for validation study with fish). Though the lactate meter is designed for use with whole blood, plasma can also be utilized by fitting values to an equation (y = 0.8202x + 0.1292, R2 = 0.9874) to obtain corrected numbers (Jerome *et al*., unpublished data).

### 2.4 Statistical Analisys

Paired student's t-test was used to evaluate for statistical differences between initial (1 minute after landing) and final temperature (prior to the release of the shark) measured at body and flank of sharks. To evaluate the potential effects of environmental temperature on changes in surface temperature, Student's t-tests were used to compare water temperature against body and flank temperature at landing and air temperature against body and flank temperature prior to release. The individual mean temperatures of twelve random points from body and flanks in the images taken were compared through one-way ANOVA. A linear regression was used to verify the relation between plasma lactate and time hooked for each animal. Linear regression was also used to verify the relation between flank temperature and lactate concentration. Normality was verified using Shapiro-Wilk test and homogeneity was verified using Levene’s test. All tests were performed with a limit of significance of 0.05, using R software for statistical computing and graphics (R Development Core Team, 2016).

## 3 Results

The average temperature (T) of body increased from 25.8°C±0.33 (S.E) at landing to 26.7°C±0.34 (S.E) right before release (n=16, Paired t-test; T=-10.488, P< 0.001) (Table 1). The average temperature (T) of flanks decreased from 31.6°C±0.36 (S.E) at landing to 30.9°C±0.38 (S.E) right before release (Paired t-test; T+=14.000 T-= -122.000, P= 0.003) (Table 2). Water temperature vs. body temperature at landing did not differed (Student's t-test; T=270.000, P= 0.835), while water temperature vs. flank temperature at landing differed (Student's t-test, T=11.520, P< 0.001). In addition, air temperature vs. body temperature prior to release differed (Student's t-test; T=196.000, P=0.011) as did air temperature vs. flank temperature prior to release (Student's t-test; T=5.167, P< 0.001).

Results showed a characteristic heat refraction from nurse shark flanks (Table 2; Figure 2). The difference in temperature was observed in all animals sampled and the flanks showed a difference in temperature of 5.8°C±0.18 (S.E.) when compared to the rest of the body at landing. The difference in temperature between flanks and body was lower prior to release (4.2°C±0.06 S.E.) (One-Way ANOVA; F=67.472, P<0.001).

| Sharks | Total length (cm) | Initial Body Temp (°C) | Final Body Temp (°C) | Air Temp (°C) | Water Temp (°C) |
|---|---|---|---|---|---|
| ♀ | 253 | 26.1±0.12 | 27.6±0.04 | 28 | 26.5 |
| ♀ | 235 | 27.5±0.15 | 28.8±0.08 | 29.6 | 27 |
| ♂ | 167 | 27.6±0.09 | 28.1±0.19 | 30.2 | 28 |
| ♀ | 261 | 28.2±0.14 | 28.9±0.04 | 30.8 | 28 |
| ♀ | 226 | 28.1±0.21 | 28.7±0.02 | 30.8 | 28 |
| ♀ | 145 | 25.2±0.06 | 25.8±0.08 | 27.3 | 25 |
| ♀ | 267 | 24.1±0.07 | 24.7±0.11 | 26.4 | 24 |
| ♂ | 246 | 24.3±0.11 | 25.1±0.07 | 26.5 | 24 |
| ♂ | 254 | 24.5±0.19 | 25.1±0.18 | 26.5 | 24 |
| ♂ | 231 | 25.1±0.08 | 26.3±0.05 | 27.9 | 25 |
| ♂ | 247 | 25.5±0.15 | 27.1±0.07 | 27 | 25 |

| | | | | | |
|---|---|---|---|---|---|
| ♂ | 264 | 25.1±0.02 | 25.9±0.12 | 27 | 25 |
| ♂ | 240 | 25.5±0.17 | 26.2±0.04 | 28.2 | 25.7 |
| ♀ | 240 | 25.3±0.12 | 26.5±0.05 | 28.2 | 25.7 |
| ♂ | 232 | 25.5±0.011 | 26.7±0.16 | 28.2 | 25.7 |
| ♂ | 243 | 25.2±0.09 | 26.3±0.09 | 28.2 | 25.7 |

**Table 1.** Initial (at lading) and Final (prior to release) body temperature (°C) of sharks after fight response in the line (mean value ± standard error). Gender, size (TL) and air – water temperature (°C) at the day of the capture are also presented.

| Sharks | Total length (cm) | Initial Flank Temp (°C) | Final Flank Temp (°C) | Air Temp (°C) | Water Temp (°C) |
|---|---|---|---|---|---|
| ♀ | 253 | 32.4±0.03 | 31.8±0.02 | 28 | 26.5 |
| ♀ | 235 | 33.4±0.09 | 32.6±0.014 | 29.6 | 27 |
| ♂ | 167 | 32.1±0.12 | 31.8±0.013 | 30.2 | 28 |
| ♀ | 261 | 33.6±0.07 | 32.9±0.08 | 30.8 | 28 |
| ♀ | 226 | 32.6±0.13 | 31.9±0.06 | 30.8 | 28 |
| ♀ | 145 | 29.5±0.03 | 28.9±0.04 | 27.3 | 25 |
| ♀ | 267 | 28.5±0.09 | 27.9±0.12 | 26.4 | 24 |
| ♂ | 246 | 29.9±0.17 | 28.1±0.16 | 26.5 | 24 |
| ♂ | 254 | 30.2±0.2 | 30.9±0.14 | 26.5 | 24 |
| ♂ | 231 | 31.1±0.04 | 31.8±0.21 | 27.9 | 25 |
| ♂ | 247 | 30.9±0.16 | 29.8±0.25 | 27 | 25 |
| ♂ | 264 | 31.2±0.08 | 30.6±0.09 | 27 | 25 |
| ♂ | 240 | 32.2±0.03 | 31.5±0.15 | 28.2 | 25.7 |
| ♀ | 240 | 31.9±0.08 | 30.5±0.21 | 28.2 | 25.7 |
| ♂ | 232 | 32.5±0.16 | 31.8±0.18 | 28.2 | 25.7 |
| ♂ | 243 | 32.9±0.07 | 31.5±0.06 | 28.2 | 25.7 |

**Table 2.** Initial (at lading) and Final (prior to release) body temperature (°C) of sharks after fight response in the line (mean value ± standard error). Gender, size (TL) and air – water temperature (°C) at the day of the capture are also presented.

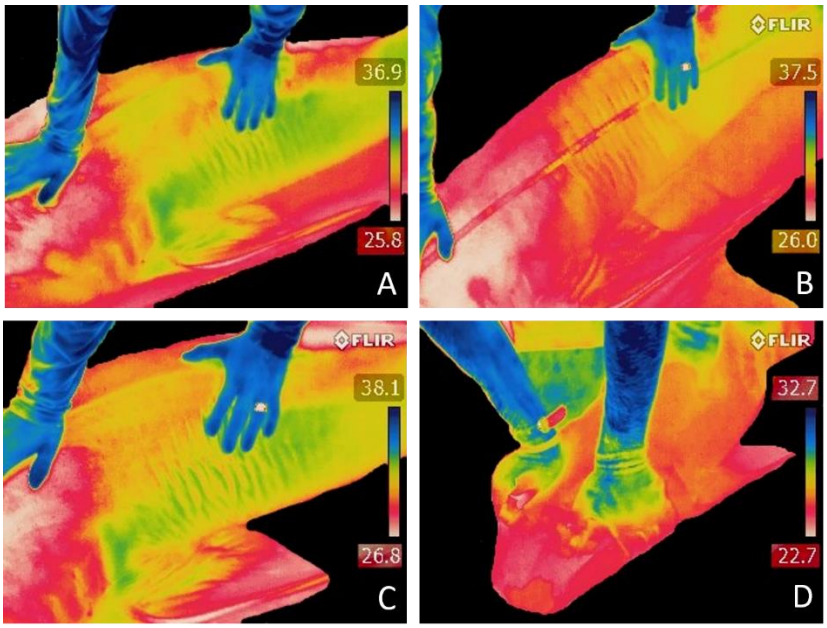



**Figure 2.** Representative infrared images of the dorsal and lateral axis of nurse sharks (A – female 1; B – male 8; C – male 11; D – female 14; see table 1 and 2 for more details) after fight response to experimental capture. The increased flank temperature (°C) at landing can be observed (yellow and green coloration). The thermal scale bar on right shows the temperature gradient.

Lactate concentration across individuals varied between 0.53 and 2.42 mM (mean 1.17 mM±0.13 S.E.). There was no significant relation between lactate concentration and time hooked (Linear Regression $R^2$= 0.000203; P= 0.958) (Figure 3), however there was a significant relation between final flank temperature and lactate concentration (Linear Regression $R^2$ = 0.370; P= 0.012; *r* = -0.214±0.07 S.E.) (Figure 4).

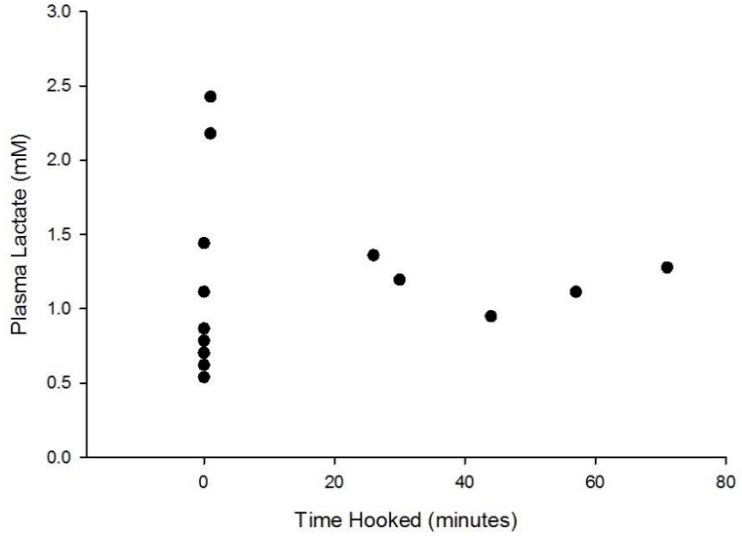



**Figure 3.** Relation between plasma lactate (mM) and time hooked (minutes) of each animal sampled.

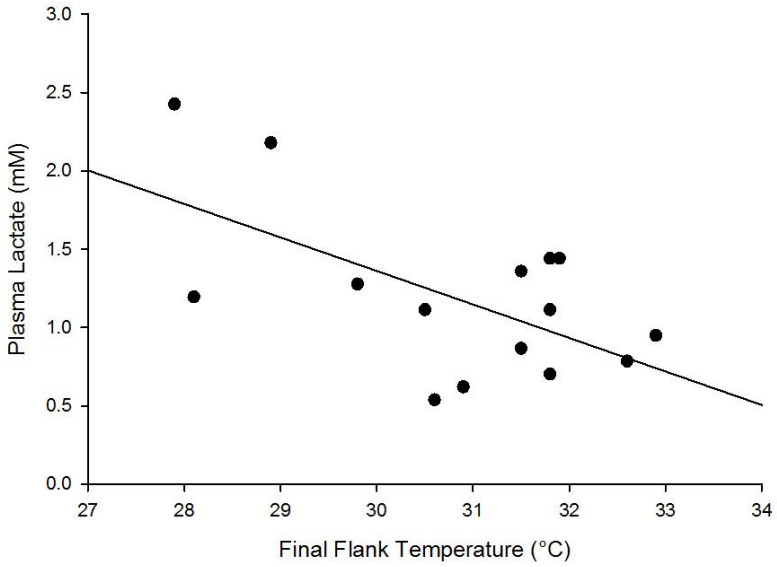



**Figure 4.** Relation between plasma lactate (mM) and final flank temperature (°C) of each animal sampled.

## 4 Discussion

Our thermal results showed a difference in flank temperature at landing and prior to release when compared to the rest of the dorsal portion of the body, indicating that there is in fact a difference in the thermal response in this region in the species. When comparing results at landing with prior to release, the

temperature of the flanks showed a decrease, indicating a relation between fighting facing capture, muscular response and rest during the restraint protocol. Also, initial flank temperature was different from water temperature as well as the final temperature in relation to the air temperature, indicating that the thermal profile registered is not as environmentally dependent as traditionally proposed for the group and may have at least partially metabolic origin. Dorsal body temperature also changed over time landed, indicating that there is a thermal stress caused by exposure to air temperature and direct solar irradiation. Lactate results showed no relation with time hooked, indicating that the stress of capture was not sufficient to generate a secondary stress response, at least in the experimental conditions applied in the present study. However, lactate concentration showed a negative relation with flank temperature prior to release, indicating that the decrease in temperature in this region is one of the factors that leads to an increase in plasma lactate.

The ability to proper respond to a stressor exposure (acute or chronic) defines the robustness of a species, making it less susceptible to post-capture mortality (Moyes *et al.* 2006). The assessment of physiological traits involved on osmoregulatory and metabolic processes is the most efficient way to determine characteristics that make species more or less susceptible, thus helping developing the proper management for each species (Skomal 2007; Skomal 2012; Wosnick *et al.* 2016). The use of thermography to determine stress response in animals is relatively new and little explored outside the veterinary field (Turner 2001; Roberto and Sousa 2014). For wild animals, thermal imagery to analyze stressful situations was already validate for the rhesus monkey (*Macaca mulatta*), which exhibit a decrease in the nasal region temperature under immediate threat (Nakayama *et al.* 2005).

In the present study, analysis of thermal images taken showed a characteristic heat dissipation on the flanks of the nurse sharks sampled. The same heat signature was not observed in the rest of the dorsal portion of the body. Studies performed with humans on muscular response also showed the same heat dissipation facing severe exercise (Krustrup *et al.* 2001). An optimized muscle response, as observed in humans, mammals and some fish species is of utmost importance on locomotory dynamics, allowing a proper fight/adaptive

response (Cooke *et al.* 2000). During muscular activity, a portion of the energy generated through metabolism is dissipated as heat. So, thermal dissipation is used as an indicator of aerobic response of red musculature during exercise (Gnaiger 1983). The flank temperature (portion of major red musculature insertion in sharks) at landing was higher when compared to the temperature prior to release, indicating that the heat dissipated may be linked to fight response facing capture. Since there was reduction in flank temperature prior to release, it is possible that the rest time during restraint/sampling has resulted in reduction of the muscular contraction.

The heat signature of the flanks was significantly higher when compared to water and air temperature. This difference was not observed in the rest of the body, indicating a weak influence of environmental temperature in the adaptive thermal response reported here, leading us to believe that the profile observed in this region likely has a metabolic (versus physical) origin. Evolutionarily, the sedentary nature of the species was suited for its lower energetic demands, making them one of the most robust species known, since most of the energy intake can be stored and used during fight response (Carlson *et al.* 2004). Thus, it is possible that the profile reported here is linked to a more pronounced/enhanced muscular response of the species facing severe exercise.

An ability to optimize muscular response implies in a delayed in the use of anaerobic fuel, reducing that way the deleterious effects of blood acidification caused by strenuous exercise (Cliff and Thurman 1984; Moyes *et al.* 2006; Awruch *et al.* 2011; Gallagher *et al.* 2014). Lower lactate levels strongly indicate a delayed anaerobic response facing capture, suggesting an efficient oxygenation (*i.e.* low CO2 contribution to respiratory acidosis) (Frick *et al.* 2012; Guida *et al.* 2016). Despite being active and efficient predators, sharks do not have a completely effective cardio-respiratory system that supplies the oxygen demands during strenuous exercise (Baldwin and Wells 1990) for that reason, some fraction of the energy needed is obtained by anaerobic metabolism and despite its importance in energy production, it may lead to deleterious effects such as metabolic acidosis and death (Cliff and Thurman 1984; Moyes *et al.* 2006; Awruch *et al.* 2011; Gallagher *et al.* 2014). The nurse sharks experimented in the present study showed low lactate levels after immediate fight response.

Also, the lactate was not influenced by the time hooked, feature that can be explained by the sedentary habits of the species, which allows greater energy and oxygen storage to be used by red musculature during fight. The same pattern was observed in the demersal shark *Mustelus antarcticus,* that did not exhibited increase in the lactate levels related to time hooked (Guida *et al*. 2016), suggesting that the primary ventilatory strategy (buccal pumping vs. ram ventilation) and stationary habit determines the ability to better respond to the stress of capture.

Finally, the lactate levels showed a negative relation with the final flank temperature, with higher levels being observed with a reduction of temperature. These results indicate a link between optimized aerobic muscular activity and delayed anaerobic activity. This feature is especially important for sharks due to the major participation of lactate in the high mortality rates exhibited by the group, thus reducing the efficiency of management protocols. A species that can maintain oxygen-dependent activity for longer will exhibit lower lactate levels being less prone to the deleterious effects of metabolic acidosis. That way, the robustness exhibit by nurse sharks facing capture may be linked not only by the lower metabolism rates and by less dependence on active ventilation, but also by the muscular response and its relation to an enhanced aerobic activity and delayed anaerobic response.

## 5 Conclusion

By analyzing the data of the nurse sharks air exposed after scientific capture, we believe that the species exhibit an optimized aerobic muscular activity complemented by a delayed anaerobic response, physiological features which were evidenced by the statistically proved relationship between flank temperature reduction concomitant with the increase in lactate in plasma. These results bring new insights of the possible mechanisms involved in the robustness of the species facing capture. Future studies should focus on measuring other stress indicators (*e.g.* urea) and metabolic fuels (*e.g*. ketone bodies) allied with thermography, aiming a better understanding of thermal gradients and vulnerability of sharks during air exposure. Our findings may help the improvement of conservation plans in a comparative perspective, since a better

understanding of features that increases the resilience at species levels can help to develop new tools (such as IRT) for fishing risk assessments.

## Literature cited

Afonso, A. S., Hazin, F. H., Carvalho, F., Pacheco, J. C., Hazin, H., Kerstetter, D. W., ... Burgess, G. H. (2011). Fishing gear modifications to reduce elasmobranch mortality in pelagic and bottom longline fisheries off Northeast Brazil. Fisheries Research, 108(2), 336-343.

Awruch, C. A., Simpfendorfer, C., Pankhurst, N. W. (2011). Evaluation and use of a portable field kit for measuring whole-blood lactate in sharks. Marine and Freshwater Research, 62(6), 694-699.

Baldwin, J., Wells, R. M. G. (1990). Oxygen transport potential in tropical elasmobranchs from the Great Barrier Reef: relationship between hematology and blood viscosity. Journal of Experimental Marine Biology and Ecology, 144(2), 145-155.

Brill, R., Bushnell, P., Schroff, S., Seifert, R., Galvin, M. (2008). Effects of anaerobic exercise accompanying catch-and-release fishing on blood-oxygen affinity of the sandbar shark (*Carcharhinus plumbeus*, Nardo). Journal of Experimental Marine Biology and Ecology, 354(1), 132-143.

Carlson, J. K., Goldman, K. J., Lowe, C. G., (2004). Metabolism, energetic demand, and endothermy. In: Biology of Sharks and Their Relatives. Carrier, J.C., Musick, J.A., Heithaus, M.C. (eds) CRC Press LLC, Boca Raton, FL, pp. 203–224.

Cilulko, J., Janiszewski, P., Bogdaszewski, M., Szczygielska, E. (2013). Infrared thermal imaging in studies of wild animals. European Journal of Wildlife Research, 59(1), 17-23.

Cliff, G., Thurman, G. D. (1984). Pathological and physiological effects of stress during capture and transport in the juvenile dusky shark, *Carcharhinus obscurus*. Comparative Biochemistry and Physiology (A), 78(1), 167-173.

Compagno, L. J. V. (2001). Sharks of The World: An Annotated and Illustrated Catalogue of Shark Species Known to Date. Vol. 2. No. 1. Food Agriculture Org.

Cooke, S. J., Schreer, J. F., Wahl, D. H., Philipp, D. P. (2000). Locomotory dynamics as indicators of stress in fish—remote measures utilizing activity transmitters. In Stress in Fish: New Directions. Proceedings of the International Congress on the Biology of Fish, University of Aberdeen, Scotland, 77-86.

Cuyler, L. C., Wiulsrød, R., Øritsland, N. A. (1992). Thermal infrared radiation from free living whales. Marine Mammal Science, 8(2), 120-134.

Ellis, J. R., McCully Phillips, S. R., Poisson, F. (2017). A review of capture and post-release mortality of elasmobranchs. Journal of Fish Biology, 90(3), 653-722.

Frick, L. H., Reina, R. D., Walker, T. I. (2010). Stress related physiological changes and post-release survival of Port Jackson sharks (*Heterodontus portusjacksoni*) and gummy sharks (*Mustelus antarcticus*) following gill-net and longline capture in captivity. Journal of Experimental Marine Biology and Ecology, 385, 29–37.

Gallagher, A. J., Serafy, J. E., Cooke, S. J., Hammerschlag, N. (2014). Physiological stress response, reflex impairment, and survival of five sympatric shark species following experimental capture and release. Marine Ecology Progress Series, 496, 207-218.

Gnaiger, E. (1983). Heat dissipation and energetic efficiency in animal anoxibiosis: economy contra power. Journal of Experimental Zoology (A), 228(3), 471-490.

Guida, L., Walker, T. I., Reina, R. D. (2016). Temperature Insensitivity and Behavioural Reduction of the Physiological Stress Response to Longline Capture by the Gummy Shark, *Mustelus antarcticus*. PLoS ONE 11(2): e0148829.

Heard, M., Van Rijn, J. A., Reina, R. D. Huveneers, C. (2014). Impacts of crowding, trawl duration and air exposure on the physiology of stingarees (family: Urolophidae). Conservation Physiology, 2, 1–14.

Hoffmayer, E. R., Parsons, G. R. (2001). The physiological response to capture and handling stress in the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*. Fish Physiology and Biochemistry, 25, 277–285.

Krustrup, P., González-Alonso, J., Quistorff, B., Bangsbo, J. (2001). Muscle heat production and anaerobic energy turnover during repeated intense dynamic exercise in humans. The Journal of Physiology, 536(3), 947-956.

Kuraoka K., Nakamura K. (2011). The use of nasal skin temperature measurements in studying emotion in macaque monkeys. Physiology and Behavior, 102, 347–355.

Mauck, B., Bilgmann, K., Jones, D.D., Eysel, U. Dehnhardt, G. (2003). Thermal windows on the trunk of hauled-out seals: hot spots for thermoregulatory evaporation? Journal of Experimental Biology, 206, 1727–1738.

McCafferty, D. J., Currie, J., Sparling, C. E. (2007). The effect of instrument attachment on the surface temperature of juvenile grey seals (Halichoerus grypus) as measured by infrared thermography. Deep Sea Research Part II: Topical Studies in Oceanography, 54(3), 424-436.

Moyes, C. D., Fragoso, N., Musyl, M. K., Brill, R. W. (2006). Predicting post release survival in large pelagic fish. Transactions of the American Fisheries Society, 135(5), 1389-1397.

Nakayama, K., Goto, S., Kuraoka, K., Nakamura, K. (2005). Decrease in nasal temperature of rhesus monkeys (*Macaca mulatta*) in negative emotional state. Physiology and Behavior, 84, 783-790

Nowack, J., Dausmann, K.H., Mzilikazi, N., 2013. Non-shivering thermogenesis in the African lesser bush baby, *Galago moholi*. Journal of Experimental Biology, 216, 3811–3817.

Pabst, D. A., McLellan, W. A., Meagher, E. M., Westgate, A. J. (2002). Measuring Temperatures and Heat Flux from Dolphins in the Eastern Tropical Pacific: is Thermal Stress Associated with Chase and Capture in the ETP-tuna Purse Seine Fishery. LJ-02-34C, pp. 1–52. National Marine Fisheries Service NOAA, La Jolla, CA, USA.

Roberto, J. V. B., de Souza, B. B. (2014). Utilização da termografia de infravermelho na medicina veterinária e na produção animal. Journal of Animal Behavior and Biometeorology, 2(3), 73-84.

Rosa, R. S., Castro, A. L. F., Furtado, M., Monzini, J., Grubbs, R. D. (2006). *Ginglymostoma cirratum*. The IUCN Red List of Threatened Species 2006:

Skomal, G. B. (2007). Evaluating the physiological and physical consequences of capture on post-release survivorship in large pelagic fishes. Fisheries Management and Ecology, 14, 81–89.

Skomal, G., Bernal, D. (2010). Physiological responses to stress in sharks. In: Sharks and Their Relatives II: Biodiversity, Adaptive Physiology, and Conservation. Jeffrey C. Carrier, John A. Musick, and Michael R. Heithaus (eds). CRC Press, Boca Raton, pp. 459-490.

Skomal, G. B., Mandelman, J. W. (2012). The physiological response to anthropogenic stressors in marine elasmobranch fishes: a review with a focus on the secondary response. Comparative Biochemistry and Physiology (A), 162, 146–155.

Tattersall, G. J., Leite, C. A., Sanders, C. E., Cadena, V., Andrade, D. V., Abe, A. S., Milsom, W. K. (2016). Seasonal reproductive endothermy in tegu lizards. Science advances, 2(1), e1500951.

Terrien J., Perret M., Aujard F. (2011). Behavioral thermoregulation in mammals: a review. Frontiers in Bioscience, 16, 1428–1444

Thompson, L. J., Brown, M., Downs, C. T., 2015. The potential effects of climate-change associated temperature increases on the metabolic rate of a small Afrotropical bird. Journal of Experimental Biology, 218, 1504–1512.

Turner, T. A. (2001). Diagnostic thermography. Veterinary Clinics of North America-Equine Practice, 17(1), 95-114.

Usamentiaga, R., Venegas, P., Guerediaga, J., Vega, L., Molleda, J., Bulnes, F. G. (2014). Infrared thermography for temperature measurement and non-destructive testing. Sensors, 14(7), 12305-12348.

Whitney, N. M., Lear, K. O., Gaskins, L. C., Gleiss, A. C. (2016). The effects of temperature and swimming speed on the metabolic rate of the nurse shark (*Ginglymostoma cirratum*, Bonaterre). Journal of Experimental Marine Biology and Ecology, 477, 40-46.

Wosnick, N., Bornatowski, H., Ferraz, C., Afonso, A., Rangel, B. S., Hazin, F. H. V., Freire, C. A. (2016). Talking to the dead: using *Post-mortem* data in the assessment of stress in tiger sharks (*Galeocerdo cuvier*) (Péron and Lesueur, 1822). Fish Physiology and Biochemistry, 1-14.

# CAPÍTULO 4

**Thermal profile in the blacktip shark (*Carcharhinus limbatus*) air exposed and implications for recreational fisheries**

**Abstract**

Fishing mortality is one of the major challenges for shark conservation. Despite the stress caused by strenuous exercise and hook injuries, the effects of water temperature and prolonged air exposure are considered the main causative agents related to the reduction of the post-release recovery capacity. During prolonged air exposure, sharks tend to experience the effects of air temperature and solar radiation. However, little is known about the consequences of this exposure on fish survival. Allied to that, the speed at which body temperatures can change, how heat is distributed across the body, and if these thermoregulatory patterns vary intra-specifically by size or life-stage also remain poorly understood. To gain insight into these knowledge gaps, the present study investigated the external body temperature profiles of blacktip sharks (*Carcharhinus limbatus)* exposed to air and solar radiation during scientific sampling. Using thermal imaging, we documented changes (ΔT=1.93±0.5˚C) in skin temperature of both adult and juvenile sharks. The observed increase was faster in juveniles (4 minutes) than in adults (up to 7 minutes). However, there was no difference between the magnitude of increase in the body surface temperature after exposure across life-stages (juveniles; ΔT=1.85±0.9˚C) (adults; ΔT=1.94±0.6˚C). A differentiation in the patterns of heat distribution in the body over time was observed, with a slower increase of temperature in the head and fins. These results suggest a higher sensitivity of the species to abrupt changes in temperature, feature that could be explained by the close relation between ecological aspects of the species (egress from nursery areas and migration) and changes in environmental temperatures. However, this physiological feature may also be responsible, at least partially, to the high mortality rates observed during capture and air exposure. We discuss these results as they relate to the challenges in the legislative context for recreational fisheries (time of air exposure during the practice) since the annual catch of this sector is now equal to or greater than the commercial sector, representing that way, a challenge for blacktip shark's conservation.

## 1 Introduction

At-vessel mortality (AVM) and Post-release mortality (PRM) are the main factors that compromise the efficiency of fishing management plans for sharks

(Ellis *et al.* 2016). Since most of the species regularly caught in commercial fishing are highly sensitive to stress caused by capture, the mortality rates are often high (Moyes *et al.* 2006; Morgan and Burgess 2007; Gallagher *et al.* 2014). Among the stressors involved with AVM and PRM are strenuous exercise associated with struggling under prolonged air exposure, with effects depending on size, sex and age, and which may vary across species (Ellis *et al.* 2016).

The concept of stress involves sub lethal consequences whose underlying mechanisms are a family of responses that reduce immunological, behavioral (*e.g.* feeding dynamics and predation avoidance), physiological (*e.g.* reproduction, metabolism and growth) (Gallagher *et al.* 2014; Hyatt *et al.* 2016), reducing the chances of survival in the long term. Furthermore, these effects may act in synergy with the environmental conditions under which fishing practices occur, for example water and air exposure; the two most relevant modulators of fish survival (Chopin and Arimoto 1995; Davis 2002; Cooke *et al.* 2003; Gingerich *et al.* 2007; Poisson *et al*. 2014). Whereas elevated water temperature increases cardiac output and metabolic/ventilatory dynamics (Prosser 1991), prolonged exposure to air usually collapses gill lamellae impairing acid-basic homeostasis (Suski *et al.* 2004) and compromise osmoregulatory processes, in turn increasing post-release mortality (Ellis *et al*. 2016).

Although several studies have elucidated the combined effects of water temperature and air exposure on fish survival (Chopin and Arimoto 1995; Davis 2002; Poisson *et al.* 2014), little is known about how air temperature and solar radiation influence the body temperature of fish during fishing events. Also, little is known about how these variables influence post-release recovery (Cicia *et al.* 2012). When a shark is pulled off water and then returned to its natural environment, the animal is exposed to abnormal temperature gradients, which may be very acute since they are normally pulled from depths of around 30 meters (Morgan and Burgess 2007). At surface, sharks are exposed to environmental temperatures that can surpass body temperature by 10-20°C (Mitchell *et al.* 2014). When returned to the water, the thermal shock may be even higher since abrupt exposure to lower temperatures (cold shock) can compromise even more the post-release recovery (Donaldson *et al.* 2008)

The blacktip shark, *Carcharhinus limbatus* (Müller  Henle 1839), is often caught in commercial fisheries by a variety of fishing gears (Morgan and Burgess

2007; Morgan and Carlson 2010; Serafy *et al.* 2012). Additionally, it is listed as game fish by the International Game Fish Association, making the species particularly vulnerable to the recreational capture. In fact, the recreational catch in the United States has approached or even surpassed the commercial capture (Fowler *et al.* 2005). Despite the specific regulation for recreational fishing for sharks (catch and release), the prolonged time hooked, the temperature gradient at which the animal is exposed when captured at greater depths, the prolonged air exposure and the thermal shock when returned to the water can reduce drastically the post-release recovery, compromising that way the measurements set by legislation to reduce the effects of this fishing sector (Arlinghaus *et al.* 2008). Additionally, the species seems to be sensitive to changes in water temperature (Heupel and Simpfendorfer 2002). In particular, changes in water temperature are believed to be a cue for this species to leave nursery grounds after their first year as well as to be a driver of migratory patterns (Heupel and Simpfendorfer 2002; Heupel and Hueter 2002; Kajiura and Tellman 2016).

Given that (a) the annual recreational catch of blacktip sharks is now the same or even higher than the commercial capture, and (b) catch-and-release is mandatory for most of the countries that held recreational fishing legislation, the understanding of how factors such as air temperature and intensity of solar radiation affect the body temperature of hooked sharks is imperative for the improvement of management plans and release protocols in the recreational sector. In this study, we used IRT to measure external body temperature profiles of blacktip sharks air exposed for approximately ten minutes. We used these data to answer the following five questions: (1) do blacktips sharks exhibit increases in external body temperature when air exposed? (2) If so, what is the rate at which the body surface temperatures changes? (3) Are fishing-related temperature shifts uniformly distributed through the body? (4) What are the effects of body size in this context? and (5) How sensitive are blacktips to airborne-induced thermal shifts, and what are the main factors affecting their recovery?

## 2 Material and methods

### 2.1 Shark Capture and Sampling

During a seven-month survey, twenty-eight blacktips (14 juveniles and 24 adults) were captured using circle hook drumlines as described in Gallagher *et al.* (2014) as part of ongoing research surveys off the coast of Miami, Florida (USA). Briefly, drumlines were deployed to soak for 1 hour before being checked for shark presence. Upon capture, sharks were secured using a custom-designed platform at the stern of the boat. Thus, sharks were completely out of the water and exposed to air, permitting thermal imaging. To promote animal welfare and vitality during sampling, a water pump was inserted into the shark's mouth and remained pumping 94.5 liters of water per minute throughout the ten-minute procedure to enable ventilation of the gills. Sharks were also sexed and total lengths measured using a standard measuring tape.

Experimental procedures and animal husbandry were approved by the University of Miami Institutional Animal Care and Use Committee (Protocol 15-238) and research permits from Florida Fish and Wildlife Conservation Commisson, Biscayne National Park and National Marine Fisheries Service.

### 2.2 Infrared Thermography

Thermal images were taken using a FLIR camera (model T420-62101), both in the first minute after landing, and just prior to the release of the shark (Figure 1). Emissivity coefficient was set at 0.98 – 0.99 units of energy according to the value established for skin in animal research. Each thermal image was analyzed using the FLIR software tools (FLIR® Systems, Inc. version 5.3.15268.1001, 2016). Mean temperatures of the dorsal axis of the sharks were calculated from 20 points of temperature measurements derived from the thermal images taken.

**Figure 1.** Thermal images being taken from an adult blacktip during scientific sampling. Photo by Alex Anstett.

For ontogenetic analysis, sharks were separated into two groups based on size and life stages according to Castro (1996): (I) TL between 105.5 – 120 cm (considered juveniles in western North Atlantic) and (II) 130 – 176 cm (considered adults in western North Atlantic).

### 2.3 Statistical Analysis

Paired student's t-Tests were used to evaluate for statistical differences between initial (1 minute after landing) and final temperature (prior to the release of the shark) measured on the dorsal surface of sharks. To evaluate the potential effects of environmental temperature on changes in body temperature, Student's t-Tests were used to compare water temperature against body temperature at landing and air temperature against body temperature prior to release.

Student's t-Tests were also used to analyze heat distribution patterns across body in intermediate time of exposure (2.5 minutes for juveniles and 4.5 minutes for adults) and prior to release (10 minutes for juveniles and adults) in two separated clusters: (1) head and fins and (2) flanks. Finally, One Way ANOVA-Test was used to evaluate for statistical differences on absolute body temperature between groups based on size classes/life stages.

Tests were performed on data from all 28 individuals grouped and analyses were computed using the R-GUI (Graphical User Interface) software (R Foundation for Statistical Computing, Vienna, Austria 2016) and statistical significance was declared at $P < 0.05$.

## 3 Results

Table 1 shows sex, total length, air temperature, and water temperature at the day of capture for each shark in each stage. The average of the 20 points established in the photos in the beginning of exposure ($T_1$), during the exposure (~5minutes; $T_{int}$) and prior to release (~10 minutes; $T_2$) is also shown in Table 1.

| Stage | Sex | Total length (cm) | $T_1$ | Tint | $T_2$ | Air T | Water T |
|---|---|---|---|---|---|---|---|
| I | ♀ | 105.5 | 24.7±0.05 | 26±0.02 | 26.8±0.03 | 26.7 | 24 |
| | ♀ | 120 | 24.6±0.08 | 25.3±0.04 | 26.4±0.04 | 26.7 | 24 |
| | ♂ | 118 | 23.8±0.12 | 24.8±0.017 | 25.7±0.14 | 23.2 | 21 |
| | ♀ | 118 | 28.2±0.04 | 29±0.05 | 29.8±0.12 | 30.3 | 28 |
| II | ♂ | 159 | 27.2±0.02 | 28.1±0.06 | 28.5±0.18 | 29 | 27 |
| | ♀ | 160 | 27.4±0.14 | 28±0.07 | 28.4±0.05 | 29 | 27 |
| | ♀ | 158 | 26.8±0.06 | 27.8±0.11 | 28.6±0.07 | 29.5 | 27 |
| | ♂ | 146 | 28.3±0.02 | 29.6±0.05 | 30.2±0.02 | 30 | 28 |
| | ♂ | 157 | 27.4±0.07 | 28.4±0.03 | 29.7±0.02 | 30 | 28 |
| | ♂ | 151 | 20.3±0.11 | 21.4±0.13 | 22.5±0.14 | 21 | 20 |
| | ♀ | 132 | 21.4±0.06 | 22.5±0.17 | 23.2±0.07 | 21 | 20 |
| | ♂ | 151 | 20.4±0.07 | 21.2±0.05 | 22.7±0.06 | 21 | 20 |
| | ♂ | 130 | 20.1±0.15 | 21.3±0.03 | 22.3±0.04 | 21 | 20 |
| | ♂ | 152 | 29.2±0.13 | 30.4±0.04 | 32.7±0.18 | 33 | 31 |
| | ♀ | 134 | 24.9±0.09 | 26.2±0.06 | 26.5±0.05 | 26.5 | 24 |
| | ♀ | 136 | 24.7±0.03 | 25.8±0.05 | 26.7±0.07 | 26.5 | 24 |

| | | | | | | |
|---|---|---|---|---|---|---|
| ♂ | 149 | 25.8±0.12 | 26.8±0.14 | 27.4±0.08 | 28.5 | 26 |
| ♀ | 176 | 26.8±0.02 | 27.9±0.05 | 28.6±0.06 | 29 | 27 |
| ♀ | 162 | 27.1±0.14 | 27.8±0.12 | 28.1±0.09 | 29 | 27 |
| ♀ | 165 | 26.5±0.06 | 27.2±0.08 | 28.2±0.12 | 29.5 | 27 |
| ♀ | 167 | 27±0.08 | 28.3±0.03 | 29.6±0.06 | 29.5 | 27 |
| ♀ | 162 | 27.2±0.17 | 28.5±0.07 | 29.2±0.12 | 29.5 | 27 |
| ♂ | 168 | 28.6±0.05 | 29.5±0.08 | 29.8±0.03 | 30.5 | 28 |
| ♀ | 171 | 24.5±0.05 | 25.80.04± | 26.4±0.08 | 26.7 | 24 |
| ♂ | 162 | 28.8±0.18 | 29.8±0.15 | 31.5±0.11 | 31 | 28 |
| ♀ | 171 | 28.5±0.02 | 29.8±0.12 | 30.2±0.05 | 31 | 28 |
| ♂ | 165 | 23.8±0.08 | 25.4±0.02 | 26.2±0.07 | 26.7 | 24 |
| ♂ | 171 | 20.7±0.13 | 21.7±0.05 | 22.8±0.15 | 21 | 20 |

**Table 1.** Body temperature (°C) of sharks at after landing (T1), during exposure ($T_{int}$) and prior to release (T2) (mean value ± standard error). Gender, size (TL), stage of maturation and air - water temperature at the day (°C) of capture are also presented.

The average temperature (T) of sharks increased from 25.5°C±0.53 (S.E) at landing to 27.4°C±0.52 (S.E) right before release (~10 minutes later) (n=28, Paired t-test; T=-19.523, P< 0.001). In addition, water temperature vs. body temperature at landing did not differed (Student's t-test; T=839.000, P= 0.505), as did not air temperature vs. body temperature prior to release (Student's t-test; T = 772.000, P = 0.676).

Despite the uniform increase in body temperature at the dorsal axis, some patterns relative to the time of exposure were observed. The increase in temperature was quicker (but not higher) in the points of muscular insertion across the body (flanks), while the increase was slower in the head and fins. There was statistical difference between the intermediate time of exposure when compared to the final time for both juveniles and adults (Paired t-test; T+= 406.000, T-= -0.000, P = <0.001).

Finally, the increase in temperature was quicker (2-4 minutes after landing) in juveniles (group I) when compared to adults (up to 7 minutes after landing) (group II) (One Way ANOVA-test; H=4.071, P=0.03), however, there was no statistical difference in the mean increase across size groups/life stages (ΔT=1.93±0.5˚C) (One Way ANOVA-test; H=6.041, P=0.110).

Thermal images from juveniles taken after landing (T1) and prior to release (T2) are presented in Figure 2 and 3, while thermal images from adults taken on T1 and T2 are presented in Figure 4 and 5. Thermal images from body regions taken in intermediate time of exposure ($T_{int}$) are presented in Figure 6.

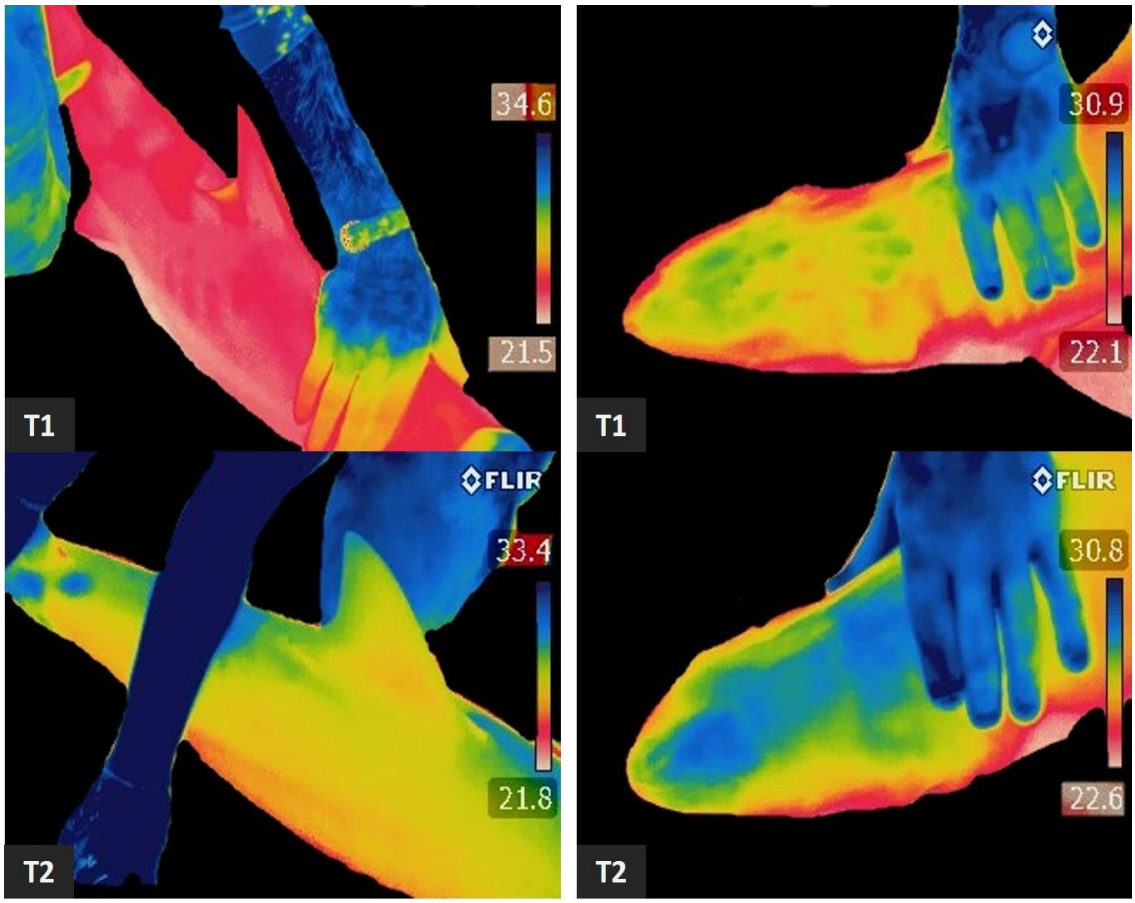



**Figure 2.** Representative infrared images of the body from two juveniles *C. limbatus* after landing (T1) and six minutes (T2) of experimental exposure to solar radiation. Body temperature according to the scale bar to the right.

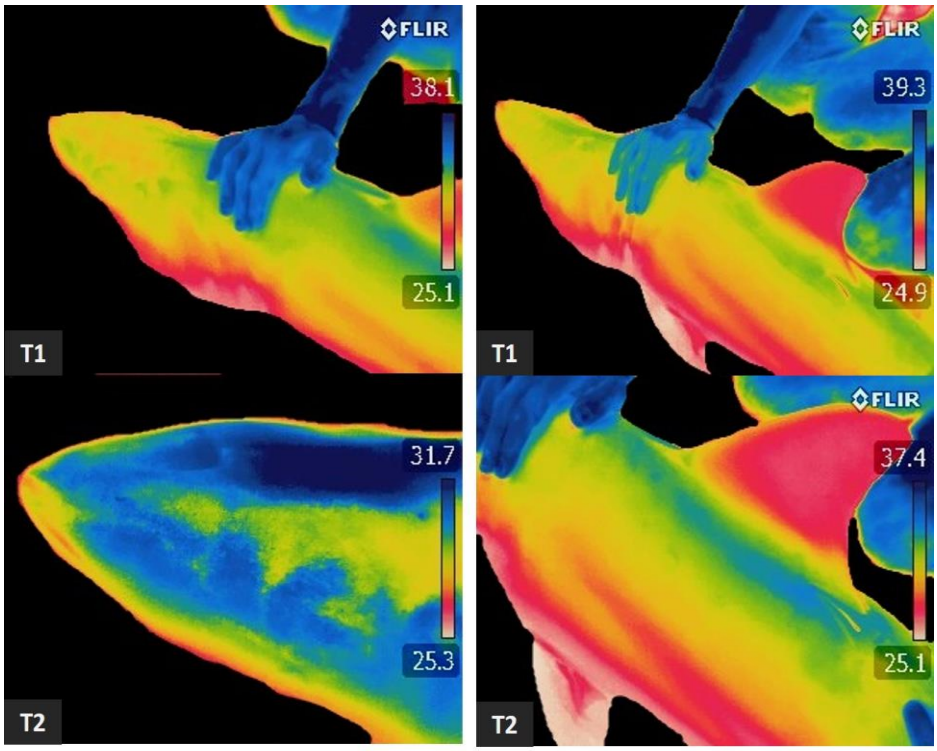



**Figure 3.** Representative infrared images of the body from two juveniles *C. limbatus* after landing (T1) and six minutes (T2) of experimental exposure to solar radiation. Body temperature according to the scale bar to the right.

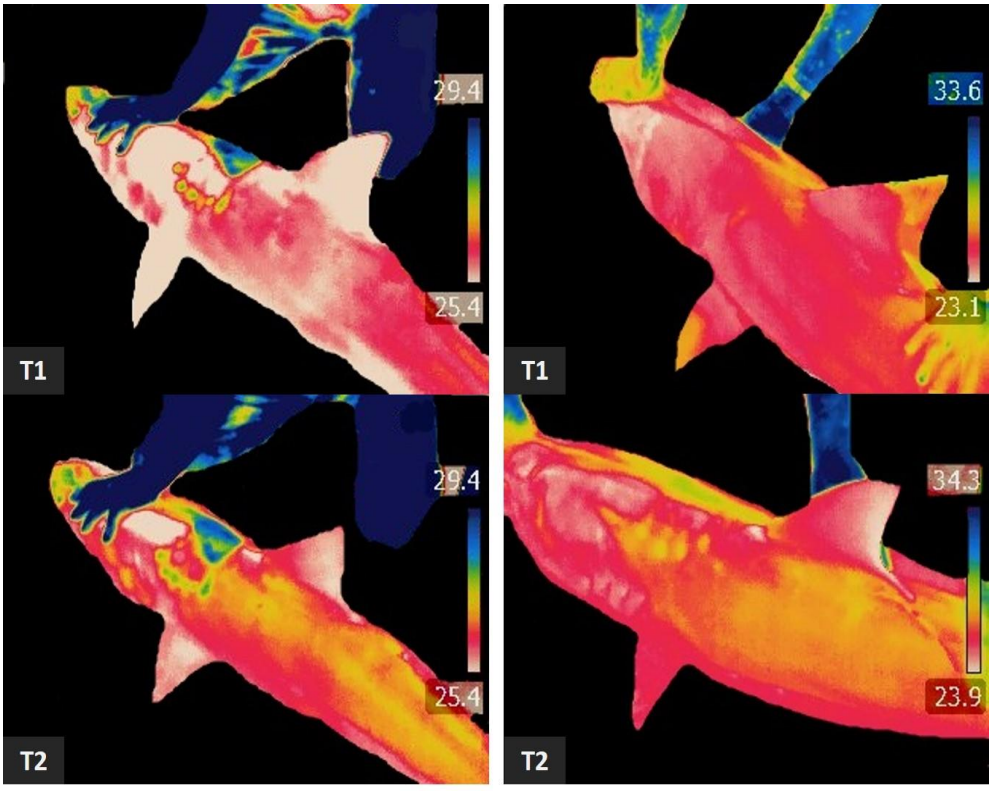



**Figure 4.** Representative infrared images of the body from two adults *C. limbatus* after landing (T1) and six minutes (T2) of experimental exposure to solar radiation. Body temperature according to he scale bar to the right.

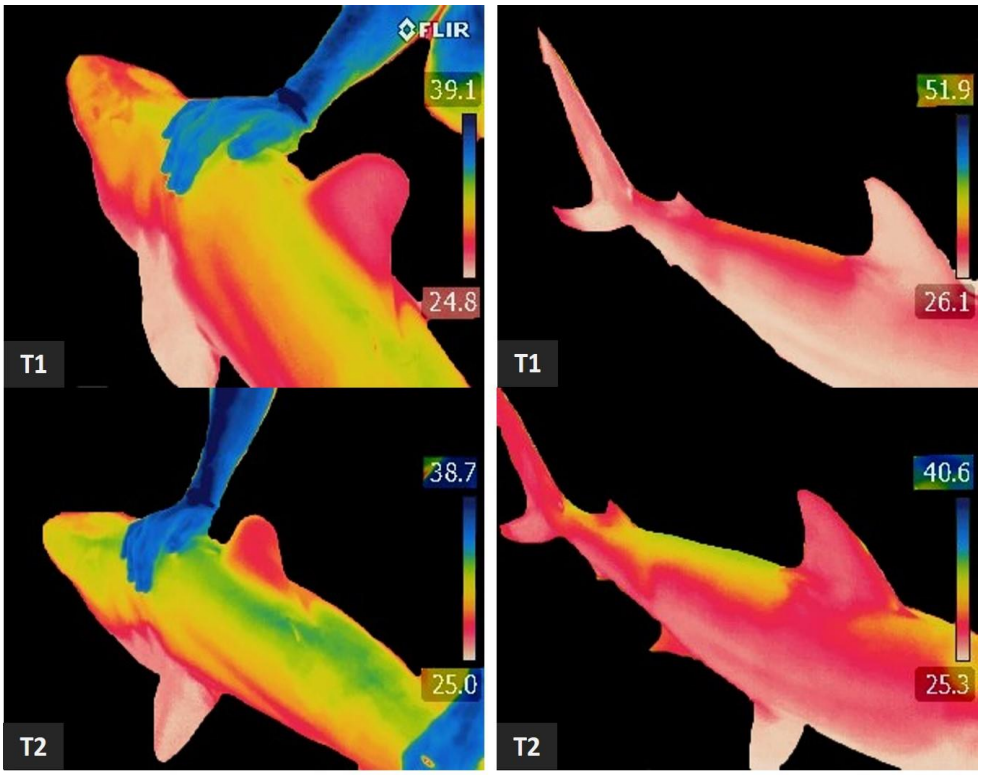



**Figure 5.** Representative infrared images of the body from two adults *C. limbatus* after landing (T1) and six minutes (T2) of experimental exposure to solar radiation. Body temperature according to the scale bar to the right.

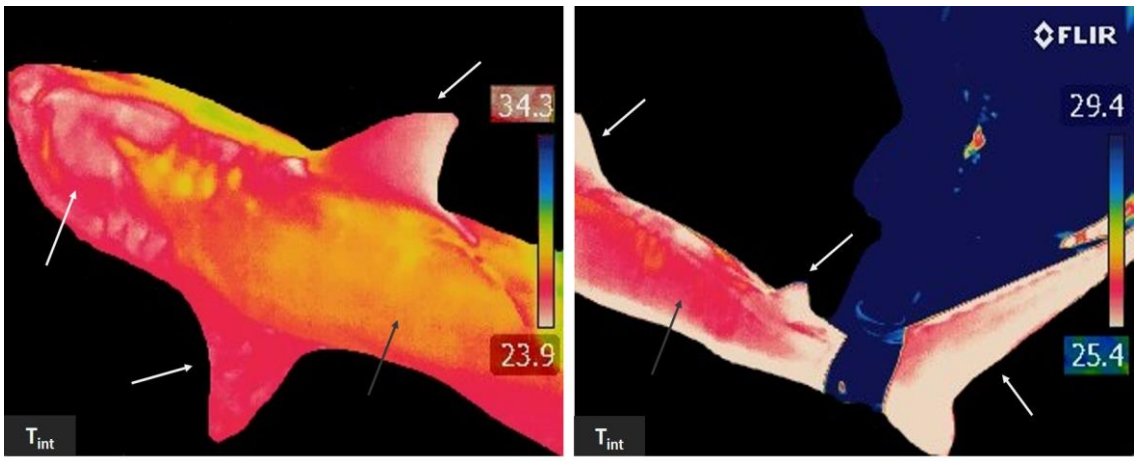



**Figure 6.** Representative infrared images body regions of *C. limbatus* taken in intermediate time of exposure ($T_{int}$). The grey arrows indicate the regions were the increase in temperature was faster (flanks) and the white arrows indicate the regions where the increase in temperature was slower (head and fins). Body temperature according to the scale bar to the right.

## 4 Discussion

Here we documented changes in skin temperature of both juvenile and adult blacktip sharks when air exposed. The observed body surface temperature

increase was more rapid in juveniles than adults. However, there was no difference in the magnitude of increase among sharks of different size or age. Despite the specific patterns of temperature distribution on the dorsal surface of the animals (flanks vs. head and fins), the amplitude in body temperature was the same.

The increase observed here suggest that blacktip sharks are sensitive to air exposure not only for the effects related to dehydration (gill collapse) and hypoxia, but also by the thermal effects caused by air temperature and solar radiation exposure. Increase in surface body temperature were expected especially due to the absence of morphological insulation mechanisms adapted to air exposure. However, the increase in sharks were more pronounced when compared to bony fish (Figure 7), being possible that this difference is related to the greater reflective capacity of leptoid scales (teleosts) when compared to placoid scales (elasmobranchs).

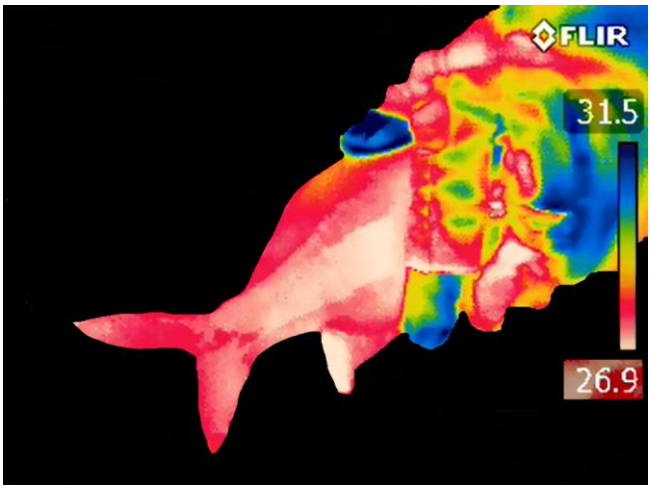



**Figure 7.** Representative infrared images of a non-identified teleost fish air exposed during scientific sampling (~6 minutes). Body temperature according to the scale bar to the right.

Although data on At-vessel mortality (AVM) and Post-release mortality (PRM) for recreational fisheries are lacking, studies performed in commercial fishing vessels indicate mortality rates between 60 and 95% for *C. limbatus* (Morgan and Burgess 2007; Morgan and Carlson 2010; Serafy *et al.* 2012). High mortality in sharks result from physiological disruption such as loss of homeostasis (Wosnick *et al.* 2016), reduction of the cardiovascular and

respiratory capacities (Randall 1982), and in general high levels of stress, still not precisely determined. Since drastic changes in body temperature directly affect thermal dynamics and metabolic rates, especially in thermal non-regulators, it is possible that the fast and steep increase in temperature recorded here is one of the physiological factors still poorly understood that reduce the survival capacity upon catch, even with quick release of the animals.

Increase in body temperature during air exposure for *C. limbatus* was first reported in the 70's through measurements of muscle temperature using thermistor probes (Carey *et al.* 1971) and significant metabolic heat gain was proposed since the animals exhibited a body temperature about 4°C higher than the surrounding environment. Most of the shark species are thought to be ectothermic, with steady-state body temperatures similar to water temperature (Carlson *et al*. 2004). Some species maintain body temperature slightly above the water temperature, trough peripheral insulation, circulatory dynamics and behavioral strategies, being considered partially endothermic (Goldman 1997; Sims *et al*. 2006; Thums et al. 2012). The concept of gigantothermy, characterized as the ability to maintain constant elevated body temperatures, low metabolic rates and peripheral insulation was proposed to leatherback turtles as a strategy to survive in lower temperatures without being endothermic (Paladino *et al.* 1990). This concept was recently proposed for whale sharks, that maintain constant elevated body core temperatures in cool deep waters (Thums *et al.* 2012; Meekan 2016). Considering smaller species such as the blacktip shark, the concept of gigantothermy theoretically does not apply, however, the increase in body temperature first described by Carey *et al.* (1971) and in the present study, points to a thermal profile that needs to be better studied, since it represents neither an ectothermy nor an endothermy.

Analysis of increase in temperature based on body regions showed differentiated patterns, that although do not have great influence in the final increase of temperature, point to thermal dynamics based on irrigation and surface area to volume ratio in the species. Changes in temperature were faster at the regions of muscle insertion (flanks) than in the head and fins (dorsal, pectoral and caudal). While the late increase in the temperature of the fins can be related to the lack of vascularization in the area, the late increase in the head

can be related to the reduced muscular insertion in the area and the physiological need for stable temperature of the brain (Wang *et al.* 2014). Despite the time of occurrence, the final increase in temperature in the dorsal region was uniform in all animals, so even with some regions exhibiting a thermal reading initially differentiated, the exposure to air temperature and solar radiation seems to have the same effects across the shark's body.

Our results also revealed that juveniles heat up more rapidly when compared to adults captured in the same area under same environmental conditions, likely due to a higher surface area to volume ratio of smaller individuals. Accordingly, we hypothesize that the influence of temperature variability and radiation exposure may be even greater in the first life stages due to the faster heating, leading to more abrupt thermal stress exposure than observed in adults, when these animals are caught and brought up in the boats, and exposed to the air. In fact, a study performed with the sablefish *Anoplopoma fimbria* showed a strong correlation between reduction in survival of animals exposed to air and their size, smaller fish being more sensitive than larger animals (Davis and Parker 2004). So, it is possible that the higher mortality rates reported for smaller/juvenile blacktip sharks may be related to the fast body heating when exposed to air temperature/solar radiation, reducing the ability to *cope* with the thermal stress generated by the abrupt change. It is important to highlight that, when these bycatch fishes are caught but later released, they may be severely disturbed by lack of oxygen during the minutes they remain out of the water, given that they do not receive branchial irrigation such as was done here during our study.

## 5 Conclusion

That way, the body's thermal sensitivity of blacktip sharks is a feature that help the animals to capture environmental cues and modulate them accordingly through changes in behavior patterns and life habits. However, the same sensitivity seems to make the species especially vulnerable to air exposure when compared to other shark's species (Wosnick *et al. in prep.*), feature that may be linked to the high mortality presented by the species facing capture. For that reason, C&R protocols in the recreational sector need to be modified. According to our data, not only water temperature and prolonged air exposure reduce the

efficiency of recreational fishing legislation, but also the air temperature and solar radiation at the day of capture. That way, it is of extreme importance the establishment of time exposed to the air during tournaments, thus reducing the effects of thermal stress and increasing the chances of post-release recovery of the species.

## Literature cited

Arlinghaus, R., Klefoth, T., Kobler, A., Cooke, S. J. (2008). Size selectivity, injury, handling time, and determinants of initial hooking mortality in recreational angling for northern pike: the influence of type and size of bait. North American Journal of Fisheries Management, 28(1), 123-134.

Carey, F. G., Teal, J. M., & Kleijn, K. (1972, February). Body temperatures of black-tip sharks *Carcharhinus limbatus*. In Deep Sea Research and Oceanographic Abstracts (Vol. 19, No. 2, pp. 179-181). Elsevier.

Carlson, J. K., Goldman, K. J., & Lowe, C. G. (2004). Metabolism, energetic demand, and endothermy. Biology of Sharks and Their Relatives, 10, 269-286.

Chopin, F. S. Arimoto, T. (1995). The condition of fish escaping from fishing gears – a review. Fisheries Research, 21, 315–327.

Cicia, A. M., Schlenker, L. S., Sulikowski, J. A., Mandelman, J.W. (2012). Seasonal variations in the physiological stress response to discrete bouts of aerial exposure in the little skate, *Leucoraja erinacea*. Comparative Biochemistry and Physiology (A), 162, 130–138.

Cooke, S. J., Suski, C. D., Barthel, B. L., Ostrand, K. G., Tufts, B. L., Philipp, D. P. (2003). Injury and mortality induced by four hook types on blue gill and pumpkin seed. North American Journal of Fisheries Management, 23, 883–893.

Davis, M. W. (2002). Key principles for understanding fish by-catch discard mortality. Canadian Journal of Fisheries and Aquatic Sciences 59, 1834–1843.

Davis, M. W., Parker, S. J. (2004). Fish size and exposure to air: potential effects on behavioral impairment and mortality rates in discarded sablefish. North American Journal of Fisheries Management, 24(2), 518-524.

Donaldson, M. R., Cooke, S. J., Patterson, D. A., Macdonald, J. S. (2008). Cold shock and fish. Journal of Fish Biology, 73(7), 1491-1530.

Ellis, J. R., McCully Phillips, S. R., Poisson, F. (2016). A review of capture and post-release mortality of elasmobranchs. Journal of fish biology, 90(3), 653-722.

Fowler, S. L.; Cavanagh, R. D.; Camhi, M.; Burgess, G. H.; Cailliet, G. M.; Fordham, S. V.; Simpfendorfer, C. A.  Musick, J. A. (2005). Sharks, Rays and Chimaeras: The Status of the Chondrichthyan Fishes. International Union for Conservation of Nature and Natural Resources. pp. 106–109, 293–295.

Gallagher, A. J., Serafy, J. E., Cooke, S. J., Hammerschlag, N. (2014). Physiological stress response, reflex impairment, and survival of five sympatric shark species following experimental capture and release. Marine Ecology Progress Series, 496, 207-218.

Gingerich, A. J., Cooke, S. J., Hanson, K. C., Donaldson, M. R., Hasler, C. T., Suski, C. D., Arlinghaus, R. (2007). Evaluation of the interactive effects of air exposure duration and water temperature on the condition and survival of angled and released fish. Fisheries Research, 86(2), 169-178.

Goldman, K. J. (1997). Regulation of body temperature in the white shark, *Carcharodon carcharias*. Journal of Comparative Physiology (B), 167(6), 423-429.

Heupel, M. R., & Simpfendorfer, C. A. (2002). Estimation of mortality of juvenile blacktip sharks, *Carcharhinus limbatus*, within a nursery area using telemetry data. Canadian Journal of Fisheries and Aquatic Sciences, 59(4), 624-632.

Heupel, M. R., & Hueter, R. E. (2002). Importance of prey density in relation to the movement patterns of juvenile blacktip sharks (*Carcharhinus limbatus*) within a coastal nursery area. Marine and Freshwater Research, 53(2), 543-550.

Hyatt, M. W., Anderson, P. A., O'Donnell, P. M. (2016). Behavioral release condition score of bull and bonnet head sharks as a coarse indicator of stress. Journal of Coastal Research, 32(6), 1464-1472.

Kajiura, S. M., & Tellman, S. L. (2016). Quantification of massive seasonal aggregations of blacktip sharks (*Carcharhinus limbatus*) in Southeast Florida. PloS one, 11(3), e0150911.

Mitchell, J. D., Collins, K. J., Miller, P. I., Suberg, L. A. (2014). Quantifying the impact of environmental variables upon catch per unit effort of the blue shark *Prionace glauca* in the western English Channel. Journal of fish biology, 85(3), 657-670.

Meekan, M. G. (2016). Will the world's biggest fish get any bigger? Limits to the size of filter-feeding fishes over the last 160 million years. Science Proceedings, 34.

Morgan, A., Burgess, G. H. (2007). At-vessel fishing mortality for six species of sharks caught in the Northwest Atlantic and Gulf of Mexico. Gulf and Caribbean Research, 19(2), 123-129.

Morgan, A., Carlson, J. K. (2010). Capture time, size and hooking mortality of bottom longline-caught sharks. Fisheries Research, 101(1), 32-37.

Moyes, C. D., Fragoso, N., Musyl, M. K., Brill, R. W. (2006). Predicting post release survival in large pelagic fish. Transactions of the American Fisheries Society, 135(5), 1389-1397.

Paladino, F. V., O'Connor, M. P., Spotila, J. R. (1990). Metabolism of leatherback turtles, gigantothermy, and thermoregulation of dinosaurs. Nature, 344(6269), 858-860.

Poisson, F., Séret, B., Vernet, A. L., Goujon, M.  Dagorn, L. (2014). Collaborative research: development of a manual on elasmobranch handling and release best practices in tropical tuna purse-seine fisheries. Marine Policy 44, 312–320.

Prosser, C. L. (1991). Environmental and Metabolic Animal Physiology. Wiley-Liss, New York.

Randall, D. J. (1982). The control of respiration and circulation in fish during exercise and hypoxia. Journal of Experimental Biology, 100, 275-88.

Serafy, J. E., Orbesen, E. S., Snodgrass, D. J., Beerkircher, L. R. Walter, J. F. (2012). Hooking survival of fishes captured by the United States Atlantic pelagic longline fishery: impact of the 2004 circle hook rule. Bulletin of Marine Science 88, 605–621.

Shiffman, D. S., Gallagher, A., Wester, J., Macdonald, C., Thaler, A. D., Cooke, S. J., Hammerschlag, N. (2014). Trophy fishing for species threatened with extinction: a way forward building on a history of conservation. Marine Policy, 50, 318-322

Shiffman, D. S., Hammerschlag, N. (2014). An assessment of the scale, practices, and conservation implications of Florida's charterboat-based recreational shark fishery. Fisheries, 39(9), 395-407

Sims, D. W., Wearmouth, V. J., Southall, E. J., Hill, J. M., Moore, P., Rawlinson, K., ... Nash, J. P. (2006). Hunt warm, rest cool: bioenergetic strategy underlying diel vertical migration of a benthic shark. Journal of Animal Ecology, 75(1), 176-190.

Suski, C. D., Killen, S. S., Cooke, S. J., Kieffer, J. D., Philipp, D. P., Tufts, B. L., (2004). Physiological significance of the weigh-in during live-release angling tournaments for largemouth bass. Transactions of the American Fisheries Society, 133, 1291–1303.

Thums, M., Meekan, M., Stevens, J., Wilson, S., Polovina, J. (2012). Evidence for behavioural thermoregulation by the world's largest fish. Journal of The Royal Society Interface, rsif20120477.

Wang, H., Wang, B., Normoyle, K. P., Jackson, K., Spitler, K., Sharrock, M. F., ... Du, R. (2014). Brain temperature and its fundamental properties: a review for clinical neuroscientists. Frontiers in Neuroscience, 8, 307.

Whitney, N. M., White, C. F., Gleiss, A. C., Schwieterman, G. D., Anderson, P., Hueter, R. E. and Skomal, G. B. (2016). A novel method for determining post-release mortality, behavior, and recovery period using acceleration data loggers. Fisheries Research, 183, 210-221.

Wosnick, N., Bornatowski, H., Ferraz, C., Afonso, A., Rangel, B. S., Hazin, F. H. V., & Freire, C. A. (2017). Talking to the dead: using *Post-mortem* data in the assessment of stress in tiger sharks (*Galeocerdo cuvier*) (Péron and Lesueur, 1822). Fish physiology and biochemistry, 43(1), 165-178.

# CAPÍTULO 5

**Physiological vulnerability of the lesser guitarfish (*Zapteryx brevirostris*), an endemic and endangered elasmobranch species to changes in salinity predicted due to climate changes.**

**Abstract**

The effects of climate changes in marine species are still poorly understood, with focus on the deleterious effects of ocean acidification on organisms with calcium carbonate skeletons. The negative effects of global warming will be even more drastic in endemic and/or threatened species, due to its limited distribution and abundance. Currently, templates of global biodiversity conservation prioritization are based on framework of ‘‘irreplaceability’’ relative to ‘‘vulnerability’’, with endemic species being an important component in the establishment of priority areas for conservation. Since vulnerability is molded by ecological and physiological traits exhibited by each species, a better understanding of the physiological responses facing immediate threats is crucial to access the plasticity and ability to cope with environmental changes. The present work aimed to access the vulnerability of an endemic and endangered guitarfish (*Zapteryx brevirostris*) to increase in salinity, a climate change factor. The animals were exposed to different salinities in order to determine osmoregulation cost by drinking rate. Our results showed that the species may be negatively affected by predicted changes in future scenarios, since the increase in drinking rate points to a higher energy allocation for osmoregulation and consequently, less metabolic energy for growth, maturation and response to stress, leading us to believe that this endemic and endangered species exhibit a high vulnerability to global warming.

## 1 Introduction

The impacts of predicted climate changes denote a growing risk, affecting all levels of biodiversity, from species to ecosystems (Sala 2006). Understanding the real effects of changes at community and ecosystem levels is a challenge, since local and global interactions between stressors (environmental degradation, pollution and bio-invasion) are poorly studied. Among the negative effects of global warming are: (1) increased disease transmission (Harvell 2002), (2) local or global extinction of less resilient species (Thomas *et al.* 2004), (3) changes in distribution patterns (Parmesan 1996), and (4) physiological and behavioral modifications, leading to a disturbance and imbalance on communities and ecosystems (Walther *et al.* 2002).

The effects of climate change on aquatic organisms are still poorly explored, with focus on the deleterious effects of ocean acidification on organisms with calcium carbonate skeletons (Kleypas *et al.* 1999; 2005; Hughes *et al.* 2003; Hoegh-Guldberg *et al.* 2007). However, the effects of acidification, increase in sea surface temperature/salinity and expansion of the minimum oxygen layer, are practically unknown for marine vertebrates (Rosa and Seibel 2008).

It is believed that acidification has a direct effect on the reduction of metabolic rates and swimming activity, leading to a significant reduction in the aerobic scope (Chin *et al.* 2010). The increase in salinity predicted, especially in coastal areas, may negatively affect species with low plasticity leading to a relocation of energy for osmoregulation, and as consequence a reduced scope for growing, maturation and immune response (Chin *et al.* 2010). The increase in sea surface temperature may have even more deleterious effects, especially in ectothermic species, which modulate its Standard and Routine Metabolism Rates according to the ambient temperature (Whitlock *et al.* 2015). Finally, the effects of an expansion of the minimum oxygen layer may also shape metabolism, daily activity and distribution patterns, since it directly affects the respiratory capacity of an individual (Whitlock *et al.* 2015). Moreover, the effects of climate changes in endemic and threatened marine species may be even more invasive, since species that are evolutionary and geographically restricted to certain areas, tend to exhibit lower adaptive capacity and reduced abundance (Malcolm *et al.* 2006; Thuiller *et al.* 2006; Calosi *et al.* 2008).

Currently, the major institutional templates of global biodiversity conservation prioritization are based on framework of ‘‘irreplaceability’’ relative to ‘‘vulnerability’’ (Chin *et al.* 2010). Based on that, the greater the number of endemic species in a region, the more biodiversity is lost if that region is lost (Calosi *et al.* 2008). The use of endemic species to determine regions where implementation of conservation plans should be prioritized is a classical approach that is now being discussed in contingency plans in the face of climate changes (Brooks *et al.* 2006). Based on the premise that endemic species may be more vulnerable not only by its reduced number but also for its lower plasticity, the creation of management plans based on ecological and physiological data is of

utmost importance, since an earlier implementation may reduce significantly the negative effects and the possible extinction of species restricted to certain areas.

The effects of climate changes in elasmobranchs still represents a huge knowledge gap. Some theoretical studies are focusing on the effects in pelagic top predators due to its importance in trophic interactions and ecosystem health (Rosa and Seibel 2008). However, for mesopredators, studies focusing in the physiological responses/effects under the predicted changes are completely unknown. Physiological studies on marine animals focusing in thermoregulation are normally held under lethal methodology, since the exposure of the animals to extreme conditions make it possible a better understanding of the physiological windows of each species (Cherry *et al.* 1977; Becker and Genoway 1979; Brown *et al.* 2016; Deslauriers *et al.* 2016).

However, for elasmobranch species, the use of lethal methodology is now out of the question, since around 25% of the species are now facing some level of threat (Dent and Clarke 2015). The need for alternative to lethal sampling makes it a great challenge the real understanding of the effects of climate change in the group (Hammerschlag and Sulikowski 2011). Fortunately, several non-lethal approaches have been validated in the past years. Allied to that, studies focusing on more realistic conditions (Clark *et al.* 2001; Sundt *et al.* 2009) are now replacing the need for extreme and lethal exposures, since the results bring more realistic physiological responses.

*Zapteryx brevirostris* is an inshore guitarfish, popularly known as the lesser guitarfish, endemic to the Southwest Atlantic, distributed from eastern Brazil to northeastern Argentina (Figueiredo 1977). It is also listed as threatened (Vulnerable) in all places of occurrence (Vooren *et al.* 2006). Despite being classified as marine, the species exhibit high tolerance to a set of salinities (Wosnick and Freire 2013), at least for 12 hours. The ability to efficiently osmoregulate in different salinities is a strong indication of an evolutionary plasticity developed by the species. Currently, the major threat to the species is overfishing (Vooren *et al.* 2006). However, most of the catches are as *bycatch* in coastal fisheries, making difficult a proper assessment of current population status, since most of the landings are unreported. The species exhibit low

commercial value, being consumed mostly by artisanal fishermen or sold as “cação” (Bornatowski *et al.* 2013) in fillets, a popular cut for elasmobranch meat in Brazil and Australia (Dent and Clarke 2015). Despite its status, there is no domestic or international legislation in place to reduce the commercial capture of the species. Since the species occupies coastal areas during all stages of life, not only the direct effects of artisanal fishing may lead to an eminent extinction, but also the increasing environmental changes observed in shallow/coastal areas.

The lesser guitarfish display high tolerance to commercial fisheries capture, being landed alive and surviving out of the water for hours (*personal observation*). For that reason, the species is a good model for non-lethal studies held in laboratory. The stress of capture can be circumvented through quarantine, guaranteeing the complete recovery of the animals, thus allowing their use in physiological studies (Wosnick and Freire 2013). For that reason, this study aimed to use the species as a model for experiments of exposure to predicted realistic conditions in order to analyze the adaptability of this endemic and threatened species under environmental alterations. We focused in one physiological challenge that the species may have on the future, an theorical increase in drinking rate caused by increase in salinity predicted in coastal areas.

## 2 Material and methods

### 2.1 Animals

Adult guitarfishes captured with gillnets were purchased from fishermen in the fish market of Matinhos (coast of Paraná, Southern Brazil). Upon arrival at the beach, specimens were carefully and quickly transferred from the boat to styrofoam boxes (56.4 cm × 38.5 cm × 37.1 cm) with laboratory ice and no water in order to prevent nitrogen poisoning, eight fish in each box. The animals were then transported by car to the laboratory in a trip of about 40 minutes (50 km). Three collecting sessions were conducted between September and November 2013 and 2014, yielding a total of 30 animals.

All international, national and institutional procedures for the maintenance and use of animals in research were followed. This project was approved by the Ethics Committee on Animal Use (CEUA) (Protocol Nº 776/2014 UFPR). Authors

had a permit from the Brazilian Environmental Agency (IBAMA/ICMBio-SISBIO #20030) to obtain and use these animals for research purposes.

## 2.2 Stomach Content – non-lethal methodology validation

Since drinking rate studies are normally held under lethal sampling or expensive essays, we created an experimental protocol for low cost/non-lethal sampling. In order to test the methodology, a dead specimen had its abdomen opened and its stomach dissected to evaluate capacity (ml) (Figure 1). To access the stomach trough trachea, a 1 ml syringe was used since its length allowed easy access to the stomach of the animal when inserted through the mouth (Figure 2). In order to standardize the collections of stomach contents, all samplings were made by the same person and only three attempts were made per animal.

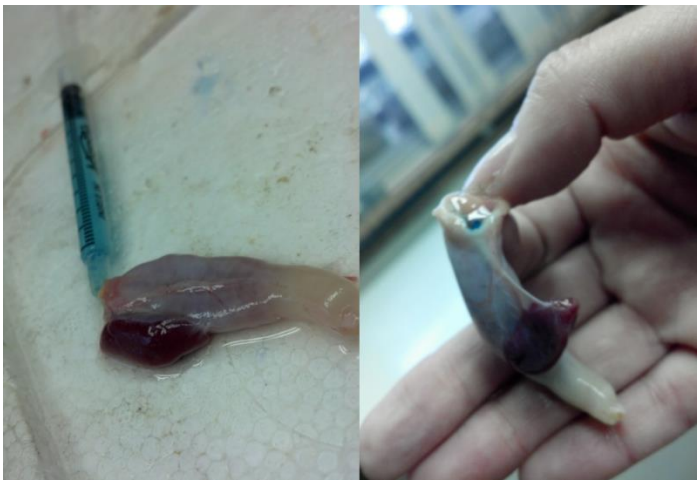


**Figure 1.** Stomach from a dead specimen dissected to access volume capacity, which is 2ml in the species. Photo by Natascha Wosnick.

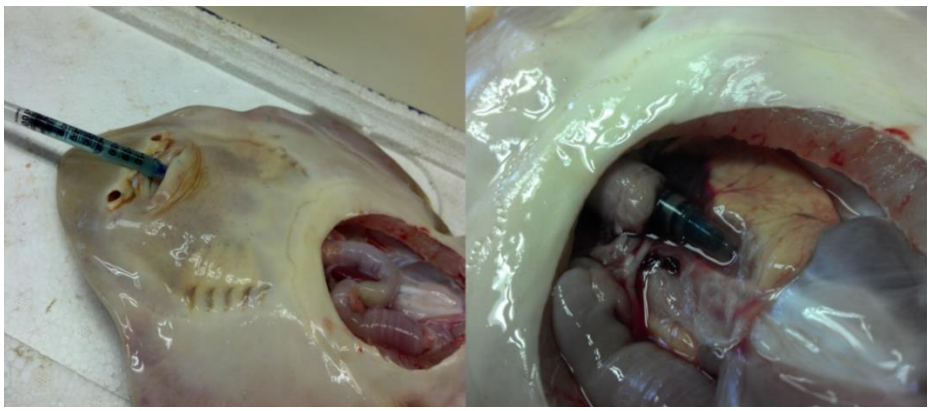


**Figure 2.** Testing the syringe size for easy access to the stomach through the animal's mouth. The stomach was removed to validate the correct positioning of the syringe. Photo by Natascha Wosnick.

## 2.3 Experiments with Salinity – Drinking Rate

In the laboratory, the animals were immediately transferred to the stock tanks (500 L), filled with seawater and a thin layer of local beach sand as substrate, under constant aeration and biological filtration, at a density of two fish/100 L. Salinity was kept at 35 psu and temperature at 20°C during the acclimation period of 24 h, and animals were not fed. After 24 h, guitarfishes were individually transferred to boxes containing 30 L of control (full-strength seawater of salinity 35 psu) or experimental (dilute seawater of salinity 25 psu) condition. Ten animals were kept in the control condition for 6 hours; ten animals were kept in salinity of 25 psu for 6 hours and ten animals were kept in salinity of 25 psu for 6 hours, then reintroduced in plastic boxes with salinity of 35 psu for 6 more hours to generate an experimental osmotic shock.

After the experimental period, the animals were anesthetized using clove oil (diluted 1:10 in 90% ethanol), with 6 mL of anesthetic solution per liter of aquarium water. After approximately 8 min, the animals displayed absence of spiracle movement and lack of response to tail grabbing/touching. The fish were then removed from the water, for blood withdraw and stomach content removal. After withdrawal, the samples (blood and stomach) were immediately centrifuged for 7 minutes at room temperature (20°C). Plasma and liquid portion of stomach content were separated and kept frozen at -20ºC until analysis at the Laboratory of Comparative Physiology of Osmoregulation in Curitiba, Paraná.

## 2.4 Plasma assays

For the salinity (drinking rate) experiments, osmolality (mOsm/kg$H_2O$) in plasma and stomach volume (µl) were assayed. Osmolality was determined using a vapor pressure osmometer (VAPRO 5520, Wescor, USA) in undiluted samples.

## 2.5 Statistical analysis

The effects of salinity in the drinking rate were evaluated through One-Way ANOVAs, and *post hoc* test of Dunn's. Shapiro–Wilk test was employed to verify normality and homogeneity of variances. Paired Student's t-tests or Wilcoxon Signed-Rank tests (when data failed Shapiro–Wilk test) were used to

verify differences in the experimental groups, pairwise. All tests were performed with limit of significance of 0.05, using Sigma Plot software version 11.0.

## 3 Results

Results showed that the control group exhibited the higher values for osmolality (964,6 mOsmo/kg$H_2O$) and stomach content (112,5µl ±15,5 S.E.). The animals exposed to salinity reduction exhibited decreased values of plasma osmolality (805,5 mOsmo/kg$H_2O$), compatible to plasma dilution under salinity reduction. The stomach content in the 25 psu group was lower (29µl ±2,7 S.E) when compared to the control group and higher when compared to the group under osmotic shock (25 > 35 psu). The plasma osmolality for the osmotic shock group (932,7 mOsmo/kg$H_2O$) was higher than the animals kept in salinity 25 psu and lower when compared to the control group. Finally, the stomach content that indicates the drinking rate under exposure, was the lowest in the group exposed to osmotic shock (9,5 µl ±2,4 S.E.) (Osmoality - One-Way ANOVA test; H= 7.38; P= 0.025) (Stomach content - One-Way ANOVA test; H= 22.9; P= <0.001) The results are showed in Figure 3.

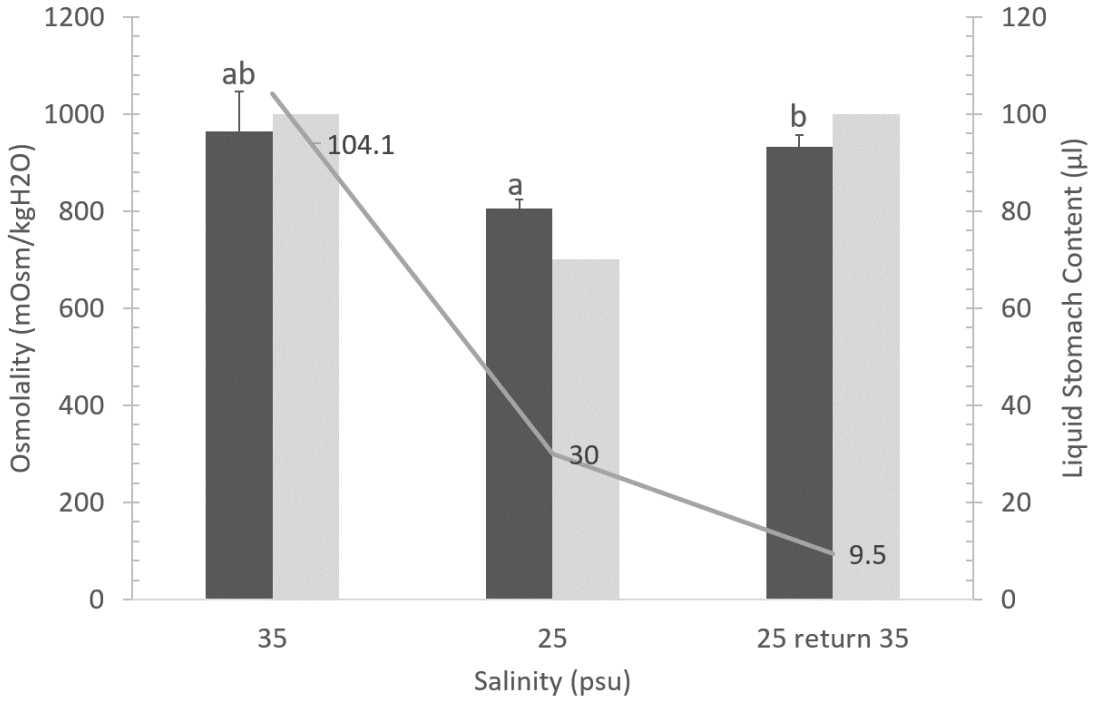



**Figure 3.** Plasma osmolality of guitarfishes exposed to different salinities for six (35 and 25 psu) and twelve (25 >35 psu) hours. The grey line represents liquid stomach content (water) in guitarfishes exposed to different salinities.The lowercase letters represent statistical differences among treatments (one-way ANOVAs, and *post hoc*

test of Dunn's - limit of significance of 0.05). The means and standard error of each group form used for the construction of the graph.

## 4 Discussion

Despite the predicted changes in elasmobranch abundance and distribution as a result of climate changes, little is known about the physiological consequences of such environmental changes. The osmotic disruptions expected by changes in salinity can lead to imminent extinction or be overcome/softened by physiological or behavioral responses (Chin *et al.* 2010). Our finding suggests that this endemic and threatened species may indeed be heavily affected by predicted environmental changes related to global warming.

It is well known that physiochemical profiles of an environment will be heavily modified by climate changes, affecting not only the environment structure and dynamics but also the species included there, which will face a set of altered environmental conditions (Fussel and Klein 2006). Alterations caused by climate changes will also modify health and recovery ability of a habitat, as well as the balance on the biological interactions within the area. Among the direct factors that will cause ecosystem imbalance by affecting the physiological response of an individual are: water temperature, ocean acidification and freshwater input (Fussel and Klein 2006; Foden *et al.* 2008; Chin *et al.* 2010). On the other hand, the indirect factors that will cause an environmental imbalance by altering habitats and processes which species depend include: ocean circulation, water and air temperature, sea level rise, severe weather and light and ultra-violet radiation (Fussel and Klein 2006; Foden *et al.* 2008; Chin *et al.* 2010).

Based on this premise and in the fact that Environmental Risk Assessments (ERA) integrate multiple variables, species and systems, taking into account the factors that alter habitats and processes is the most important strategy for proper contingency plans (Fussel and Klein 2006; Chin *et al.* 2010). On the other hand, in individual/physiological risk assessments (determine vulnerability at species level) direct factors such as water temperature, salinity and oxygen concentration are the main features to be analyzed (Chin *et al.* 2010). Since the urge for protective measurements are based on occurrence of endemic and threatened species, a complete physiological profile to define the realistic vulnerability of the species in question becomes crucial, since it is possible to

determine the real impacts that environmental changes - especially those caused by climatic changes - may have on the species and consequently on the environment in which the species is inserted.

Problematic of increased drinking rate

The drinking behavior is an essential component of osmoregulatory strategy for aquatic organisms, since it allows the proper water intake to maintain the homeostatic balance (Anderson and Hazon 2002). In mammals, while a minimal (1-2%) reduction in cellular fluid volume is enough to induce a drinking response, the reduction on extracellular fluid needs to be higher (8-10%) to induce thirst (Fitzsimons 1998). However, the same does not apply for most of the aquatic vertebrates, such as elasmobranchs, which have dipsogenic mechanisms activate more rapidly when an imbalance on extracellular fluid is detected (Takei 2000).

In hyperosmotic environments, water is constantly lost and osmoregulators drink water to maintain ionic and water balance (Oide and Utida 1968). This strategy is performed by teleost fish, and the drinking behavior in the group is well studied (Perrott *et al.* 1992 Fuentes and Eddy 1997). It has been proposed that most of the elasmobranch's species are iso or hyperosmotic in marine environment, so this strategy does not apply (in theory, sharks and batoids would actively gain water from the surrounding environment and for that reason, the drinking behaviors would not be necessary). However, the growing interest in elasmobranch physiology has led to significant discoveries, such as (1) a hypo-osmotic profile in many species when in seawater (Haywood 1973; Cooper and Morris 1998), (2) an euryhalinity capacity more common than expected for the group (Burger 1965; Goldstein and Forster 1971; Hazon and Henderson 1984; Wosnick and Freire 2013) and (3) compensatory strategies being used for sharks and batoids that were only described for bony fish in the past (Anderson and Hazon 2002).

In a changing environment, with predicted increase in seawater salinity, it is expected that compensatory strategies, such as drinking behavior, would be more necessary not only for species who already do it, but also by species that previously did not need such compensatory control, like many elasmobranchs. Despite being a simple behavior, intake of salt water implies a greater ionic

regulation and as consequence, a large amount of metabolic energy being relocated for it (Kirschner 1993; Ballantyne and Robinson 2010).

Our results showed an increase in water content in the stomach of the animals exposed to higher salinity (35 psu). In such salinity condition, the species is hypo-osmotic (Wosnick and Freire 2013), so the water content collected, can in fact, be used as indicator of drinking behavior, validating not only the hypothesis of a more spread drinking behavior in the group that the expected, but also the non-lethal and low-cost methodology tested. It may seem unambitious to use such low salinity when compared to lethal limits often tested, however, an increase of 5 psu (when considering the average salinity in the area where the animals were caught – 30 psu), represent a realistic osmotic challenge for future scenarios of climate changes. Even with values as low as the one used in the present study, the species showed an intake well above the expected, leading us to believe that with the predicted salinity changes, this endemic and threatened species would possibly add the behavior in its osmoregulatory strategy, modifying that way, its metabolic profile by spending more energy on osmoregulation processes than the regular and as consequence, reducing the amount of energy spent with growth, maturation and ability to *cope* with stress.

This may be an enormous problem for conservation plans for the species based on management strategies, such as compensatory release by artisanal fishermen, since with a significant part of the metabolic energy being relocated to osmoregulation processes, the species could reduce its resistance and robustness, dying before being landed, as occurs with other guitarfish's species.

## 5 Conclusion

Adaptive capacity may be measured by four attributes, reflecting the ability to accommodate change: (1) trophic specificity, (2) immobility, (3) physical or chemical intolerance and (4) latitudinal range. Sharks and rays with a specialist diet, which exhibit site fidelity and no migratory behavior, lower tolerance to fluctuations in abiotic conditions (stenohaline/stenothermic) and have a narrow range (such as endemic species) are much more susceptible to climate chance effects. Since *Zapteryx brevirostris* fits in most of the vulnerability categories - selective diet based on substrate preference; sedentary nature; and endemic occurrence, plus the threatened status molded by overfishing- it is plausible to

infer that the species in fact exhibit a high degree of rigidity that will negatively affect its ability to adapt to predicted changes in its areas of occurrence.

## Literature cited

Anderson, W. G., Takei, Y., Hazon, N. (2002). Osmotic and volaemic effects on drinking rate in elasmobranch fish. Journal of Experimental Biology, 205(8), 1115-1122.

Ballantyne, J. S., Robinson, J. W. (2010). Freshwater elasmobranchs: a review of their physiology and biochemistry. Journal of Comparative Physiology B, 180(4), 475-493.

Becker, C. D., Genoway, R. G. (1979). Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. Environmental Biology of Fishes, 4(3), 245-256.

Bornatowski, H., Braga, R. R., Vitule, J. R. S. (2013). Shark mislabeling threatens biodiversity. Science, 340(6135), 923-923.

Brooks, T. M., Mittermeier, R. A., da Fonseca, G. A., Gerlach, J., Hoffmann, M., Lamoreux, J. F., ...Rodrigues, A. S. (2006). Global biodiversity conservation priorities. Science, 313(5783), 58-61.

Brown, L. R., Komoroske, L. M., Wagner, R. W., Morgan-King, T., May, J. T., Connon, R. E., Fangue, N. A. (2016). Coupled Downscaled Climate Models and Ecophysiological Metrics Forecast Habitat Compression for an Endangered Estuarine Fish. PloS One, 11(1), e0146724.

Burger, J. W. (1965). Roles of the rectal gland and the kidneys in salt and water excretion in the spiny dogfish. Physiological Zoology, 38, 191–196.

Calosi, P., Bilton, D. T., Spicer, J. I. (2008). Thermal tolerance, acclimatory capacity and vulnerability to global climate change. Biology Letters, 4(1), 99-102.

Cherry, D. S., Dickson, K. L., Cairns Jr, J., Stauffer, J. R. (1977). Preferred, avoided, and lethal temperatures of fish during rising temperature conditions. Journal of the Fisheries Board of Canada, 34(2), 239-246.

Chin, A., Kyne, P. M., Walker, T. I., McAuley, R. O. R. Y. (2010). An integrated risk assessment for climate change: analyzing the vulnerability of sharks and rays on Australia's Great Barrier Reef. Global Change Biology, 16(7), 1936-1953.

Clark, J. R., Bragin, G. E., Febbo, E. J., Letinski, D. J. (2001). Toxicity of physically and chemically dispersed oils under continuous and environmentally realistic exposure conditions: Applicability to dispersant use decisions in spill response planning. In International Oil Spill Conference, 2, 1249-1255. American Petroleum Institute.

Cooper, A. R., Morris, S. (1998). The blood respiratory, haematological, acid–base and ionic status of Port Jackson shark, Heterodontus portusjacksoni, during recovery from anesthesia and surgery: a comparison with sampling by direct caudal puncture. Comparative Biochemistry and Physiology (A), 119A, 895–903.

Dent, F., Clarke, S. (2015). State of the global market for shark products. FAO Fisheries and Aquaculture Technical Paper, 590.

Deslauriers, D., Heironimus, L., Chipps, S. R. (2016). Lethal Thermal Maxima for Age-0 Pallid and Shovelnose Sturgeon: Implications for Shallow Water Habitat Restoration. River Research and Applications.

Figueiredo J. L. (1997). Manual de peixes marinhos do sudeste do Brasil. I. Introdução: Cações, raias e quimeras. São Paulo: Museu de Zoologia da Universidade de São Paulo.

Fitzsimons, J. T. (1979) The Physiology of Thirst and Sodium Appetite, pp. 1–121. Cambridge: Cambridge University Press.

Foden, W., Mace, G., Vie, J. C. *et al.* (2008). Species susceptibility to climate change impacts. In: The 2008 review of the IUCN Red List of Threatened Species. (eds) Vie, J. C., Hilton-Taylor, C., Stuart, S. N., IUCN, Gland.

Fuentes, J., Eddy, F. B. (1997). Drinking in marine, euryhaline and freshwater teleost fish. In Ionic Regulation in Animals: A Tribute to Professor WTW Potts (pp. 135-149). Springer Berlin Heidelberg.

Fussel, H. M., Klein, R. J. T. (2006). Climate change vulnerability assessments: an evolution of conceptual thinking. Climatic Change, 75, 301–329.

Goldstein, L.  Forster, R. P. (1971). Osmoregulation and urea metabolism in the little skate, Raja erinacea. American Journal of Physiology, 220, 742–746.

Hammerschlag, N., Sulikowski, J. (2011). Killing for conservation: the need for alternatives to lethal sampling of apex predatory sharks. Endangered Species Research, 14(2), 135-140.

Harvell, C.D. (2002). Climate warming and disease risks for terrestrial and marine biota. Science, 296, 2158–2162 2

Haywood, G. P. (1973). Hypo-osmotic regulation coupled with reduced metabolic urea in the dogfish Poroderma africanum: an analysis of serum osmolarity, chloride, and urea. Marine Biology, 23(2), 121-127.

Hazon, N.  Henderson, I. W. (1984). Secretory dynamics of 1α-hydroxycorticosterone in the elasmobranch fish, *Scyliorhinus canicula*. Journal of Endocrinology, 103, 205–211.

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., ...  Knowlton, N. (2007). Coral reefs under rapid climate change and ocean acidification. Science, 318(5857), 1737-1742.

Hughes, T. P., Baird, A. H., Bellwood, D. R., Card, M., Connolly, S. R., Folke, C., ...  Lough, J. M. (2003). Climate change, human impacts, and the resilience of coral reefs. Science, 301(5635), 929-933.

Kleypas, J. A., Buddemeier, R. W., Archer, D., Gattuso, J. P., Langdon, C., Opdyke, B. N. (1999). Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. Science, 284(5411), 118-120.

Kleypas, J. A., Feely, R. A., Fabry, V. J., Langdon, C., Sabine, C. L., Robbins, L. L. (2005). Impacts of ocean acidification on coral reefs and other marine calcifiers: a guide for future research. In Report of a workshop held, 18, pp. 20.

Kirschner, L. B. (1993). The energetics of osmotic regulation in ureotelic and hypoosmotic fishes. Journal of Experimental Zoology, 267, 19–26.

Malcolm, J. R., Liu, C., Neilson, R. P., Hansen, L., Hannah, L. E. E. (2006). Global warming and extinctions of endemic species from biodiversity hotspots. Conservation biology, 20(2), 538-548.

Oide, H., Utida, S. (1968). Changes in intestinal absorption and renal excretion of water during adaptation to sea-water in the Japanese eel. Marine Biology, 1, 172–177.

Parmesan, C. (1996). Climate and species range. Nature, 382, 765–766.

Perrott, M. N., Grierson, C. E., Hazon, N., Balment, R. J. (1992). Drinking behaviour in sea water and fresh water teleosts, the role of the renin-angiotensin system. Fish Physiology and Biochemistry, 10(2), 161-168.

Rosa, R., Seibel, B. A. (2008). Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator. Proceedings of the National Academy of Sciences, 105(52), 20776-20780.

Sala, E. (2006). Top predators provide insurance against climate change. Trends in Ecology and Evolution, 21(9), 479-480.

Sundt, R. C., Meier, S., Jonsson, G., Sanni, S., Beyer, J. (2009). Development of a laboratory exposure system using marine fish to carry out realistic effect studies with produced water discharged from offshore oil production. Marine pollution bulletin, 58(9), 1382-1388.

Takei, Y. (2000). Comparative physiology of body fluid regulation in vertebrates with special reference to thirst regulation. Japanese Journal of Physiology, 50, 171–186.

Thomas, C. D., Cameron, A., Green, R. E., Bakkenes, M., Beaumont, L. J., Collingham, Y. C., ... & Hughes, L. (2004). Extinction risk from climate change. Nature, 427(6970), 145-148.

Thuiller, W., Midgley, G. F., Hughes, G. O., Bomhard, B., Drew, G., Rutherford, M. C., Woodward, F. (2006). Endemic species and ecosystem sensitivity to climate change in Namibia. Global Change Biology, 12(5), 759-776.

Vooren, C.M., Lamónaca, A.F., Massa, A. Hozbor, N. (2006). *Zapteryx brevirostris*. The IUCN Red List of Threatened Species 2006: Downloaded on 18 November 2016.

Walther, G. R., Post, E., Convey, P., Menzel, A., Parmesan, C., Beebee, T. J., ... & Bairlein, F. (2002). Ecological responses to recent climate change. Nature, 416(6879), 389-395.

Whitlock, R. E., Hazen, E. L., Walli, A.,Farwell, C., Bograd, S. J., Foley, D. G., Castleton, M., Block, B. A. (2015). Direct quantification of energy intake in an apex marine predator suggests physiology is a key driver of migrations. Science Advances 1(8):e1400270–e1400270.

Wosnick, N., Freire, C. A. (2013). Some euryhalinity may be more common than expected in marine elasmobranchs: The example of the South American skate *Zapteryx brevirostris* (Elasmobranchii, Rajiformes, Rhinobatidae). Comparative Biochemistry and Physiology (A), 166(1), 36-43.

# CAPÍTULO 6

**Rapid warming of the cephalofoil in great hammerhead sharks (*Sphyrna mokarran*) and potential for thermal accommodation capacity**

**Abstract**

Hammerhead sharks (family Sphyrnidae) are known to exhibit a suite of unique morphological and physiological modifications, shaped by millions of years of evolution. Among them, the cephalofoil may be the most significant, allowing greater maneuverability, prey capture and increased sensory capacity. *Sphyrna mokarran* frequently enters shallow warm waters from offshore colder waters for hunting where they are quickly exposed to temporary increases in water temperature. Physiological modifications apparently related to the use of such inshore environments are observed in the family, including the ability to tan and modify retinal composition as protective measures against high UV incidence. Employing infrared technology, here we documented rapid (< 2 minutes) warming in the cephalofoil surface ($\Delta T=3.1\pm0.56^{\circ}C$) of *S. mokarran* experimentally exposed to air and solar radiation. This finding suggests the potential for thermal accommodation capacity of the cephalofoil surface via rapid heat energy absorption. We hypothesize this serves to protect the electrosensory system from overexcitement due to voltage changes that would otherwise occur due to rapid changes in temperature when this species enters warmer surface or shallow waters. This may be yet another specialization permitting great hammerheads to effectively exploit warm waters under high water temperatures and solar radiation. We further consider how this proposed thermal accommodation feature in great hammerheads may be potentially disturbed by predicted future climate change scenarios of increased ocean temperature and reduced penetration of solar radiation in the water column.

## 1 Introduction

The hammerhead sharks (Sphyrnidae) are among the most recently evolved, diverging from the Carcharhinid sharks ~10-20 million years ago (Naylor 1992). Distinguishing these species are a series of behavioral, morphological and physiological specializations (Gallagher *et al.* 2014a). Hammerheads are most identifiable by their cephalofoil (“hammer”), which appears to endow these sharks with enhanced foraging and sensory capabilities (McComb *et al.* 2009). This includes an increased capacity to perform rapid tight maneuvers during prey capture (Nakaya 1999; Roemer *et al.* 2016), subjugate and immobilize prey (Chapman and Gruber 2002), greater visual acuity (Tester 1983), increased

chemoreception (Tricas *et al.* 2009), and enhanced magnetic and electrical reception (Brown 2003; Kajiura and Forni 2003). These traits are likely enabled through enlarged telencephalons and cerebellums in the brain, and the highest levels of cerebellar foliation measured in any shark (Yopak *et al.* 2007; Yopak 2009, 2012).

Hammerhead sharks are among the most fascinating and threatened animals on the planet attracting tremendous attention from public citizens, wildlife managers, policy makers and the mass-media. Species that have a high degree of adaptive specialization are more vulnerable when compared to generalist species (Gallagher *et al.* 2014a). The most recent theory on hammerhead shark's evolution discuss the role of the cephalofoil size in an adaptive perspective where species with expanded cephalofoil are considered basal within the family (Lim *et al.* 2010). Coincidentally, the four less derived species (*Eusphyra blochii, Sphyrna mokarran, Sphyrna zygaena and Sphyrna lewini*) are also the most critically endangered (IUCN 2016). So, despite the clear advantage in the search and capture of prey ensured by the cephalofoil, it is believed that the morphological changes observed in hammerhead sharks are responsible for the lower resistance facing capture exhibited by the species.

*Sphyrna mokarran*, the great hammerhead, is a coastal-pelagic/semi-oceanic tropical shark that occurs both inshore and offshore waters (Compagno 2001; Graham *et al.* 2016). The maximum size for this species is reported as 550 to 610 cm (Compagno 2001). Great hammerheads exhibit quick burst swimming (Gallagher *et al.* 2014b; Roemer *et al.* 2006), a behavior also observed in other specialist predators, such as crocodiles (Bennett *et al.* 1985) and cheetahs (Schaller 1968). Anatomical and physiological adaptations permit such burst behavior, including differential muscle organization, ability to deal with anaerobic metabolism and increased lactate levels, as well as specialized body shape (Bennett *et al.* 1985; Williams and Stuarty 1999).

Recent research has revealed that this migratory coastal-pelagic/semi-oceanic species will temporarily venture from offshore waters into coastal shallow environments (<1.5 meters in depth) characterized by relatively high water temperature and low oxygen concentration in order to capture prey (Roemer *et al.* 2016). In such shallow waters, solar radiation is able to penetrate to the bottom

and heat the entire water column, reaching temperatures near 38°C (Weber 2007). To exploit these environments, great hammerheads employ a prey-capture technique involving burst swimming at tight turning angles while grasping prey and a post-predation recovery period whereby the shark maintains head-first orientation into the current that may facilitate respiration and prey consumption (Roemer *et al.* 2016).

In this study, we use thermal imagery to document rapid temperature increases in the cephalofoil surface of eleven great hammerhead sharks experimentally exposed to solar radiation. We hypothesize that measured temperature increases of the cephalofoil functions to protect the electro-sensory system (ampullae of Lorenzini) against overexcitement when temporarily entering warmer waters. This thermal capacity feature may be yet another specialization exhibited by this species for utilizing warm shallow or surface waters during hunting. We discuss this finding in terms of predicted future climate change scenarios of increased water temperature and decreased solar radiation, where such a thermal capacity specialization may become an evolutionary trap.

## 2 Material and methods

### 2.1 Animals

Eleven *S. mokarran* were captured using drumlines as part of ongoing research surveys. Total length (TL) and gender were determined for each animal (Table 1). The animals were removed from the water and placed on a boat-attached platform. A water pump was inserted into the animal's mouth and remained pumping 94.5 liters of water per minute throughout the six-minute sampling procedure to enable ventilation of the gills and ensure survival.

| Sharks | Total length (cm) | Temp (°C) first minute | Temp (°C) second minute | Air Temp (°C) | Water Temp (°C) |
|---|---|---|---|---|---|
| Female 1 | 253 | 24.1± 0.09 | 26.4± 0.20 | 27.6 | 25 |
| Female 2 | 328 | 23.5± 0.11 | 25.3± 0.14 | 26.7 | 24 |
| Female 3 | 200 | 25± 0.12 | 32± 0.06 | 34 | 32 |

| | | | | | |
|---|---|---|---|---|---|
| Female 4 | 251 | 20.3± 0.21 | 25.3± 0.08 | 30 | 28 |
| Female 5 | 293 | 27.1± 0.09 | 29.4± 0.10 | 30.3 | 28 |
| Male 1 | 259 | 24.5± 0.14 | 27.7± 0.21 | 28.5 | 26 |
| Male 2 | 267 | 19.2± 0.13 | 24.2± 0.18 | 23.2 | 21 |
| Male 3 | 269 | 18.8± 0.12 | 23.0± 0.11 | 21 | 20 |
| Male 4 | 264 | 25.8± 0.07 | 27.0± 0.08 | 26.7 | 24 |
| Male 5 | 340 | 24.5± 0.14 | 26.6 ± 0.21 | 26.7 | 24 |
| Male 6 | 284 | 23.2± 0.10 | 25.6± 0.13 | 26.7 | 24 |

**Table 1.** Cephalofoil's temperature (°C) of sharks at the first and second minute after landing (mean value ± standard error). Gender, size (TL) and air - water temperature at the day (°C) of capture are also presented.

### 2.2 Infrared Thermography

Thermal images were taken using a FLIR camera (model T420-62101 Wilsonville, Oregon, EUA) at landing of the shark and at 2 minute intervals thereafter. The relative humidity, air temperature and distance of the animal were calibrated on the camera according to the conditions at the day. Emissivity setting used was 0.98 – 0.99. Each thermal image was analyzed using the FLIR software tools (2015). Mean temperatures of the cephalofoil of the sharks were calculated from 49 points of temperature measurements derived from the thermal images taken at landing and each 2 minutes sampling interval.

Experimental procedures and animal husbandry were approved by the University of Miami Institutional Animal Care and Use Committee (Protocol 15-238) and research permits from Florida Fish and Wildlife Conservation Commission, Biscayne National Park and National Marine Fisheries Services.

### 2.3 Statistical analysis

Paired student's t-Test was used to evaluate for statistical differences between initial (1 minute after landing) and final temperature (2 minutes after landing) measured on the cephalofoil surface of sharks. To evaluate the potential

effect of water versus air temperature on changes in cephalofoil temperature, Student's t-Tests were also used to compare water temperature against cephalofoil temperature at landing (first minute) and air temperature against cephalofoil temperature after two minutes of experimental exposure to solar radiation (second minute) (Sigma Plot 11.0 Dunda Software LTD, Germany 2016). Tests were performed on data from all 11 individuals grouped and analyses were computed using the R-GUI (Graphical User Interface) software (R Foundation for Statistical Computing, Vienna, Austria 2016) and statistical significance was declared at $P < 0.05$.

## 3 Results

Across 11 animals, we documented a cephalofoil surface average temperature increase of 3.1±0.56˚C per-minute, while the temperature across the rest of the body changed less than 1.0±0.37˚C during the same time period (Figure 1A). Analysis of the thermal images of the cephalofoil taken at the first and second minute are presented in Figure 1B-F and Figure 2A-F. Across 11 individuals, cephalofoil temperatures measured at the second minute after landing was significantly higher than at 1 minute after landing (Paired t-test; $t=-6.070$, $P = <0.001$) (Figure 3).

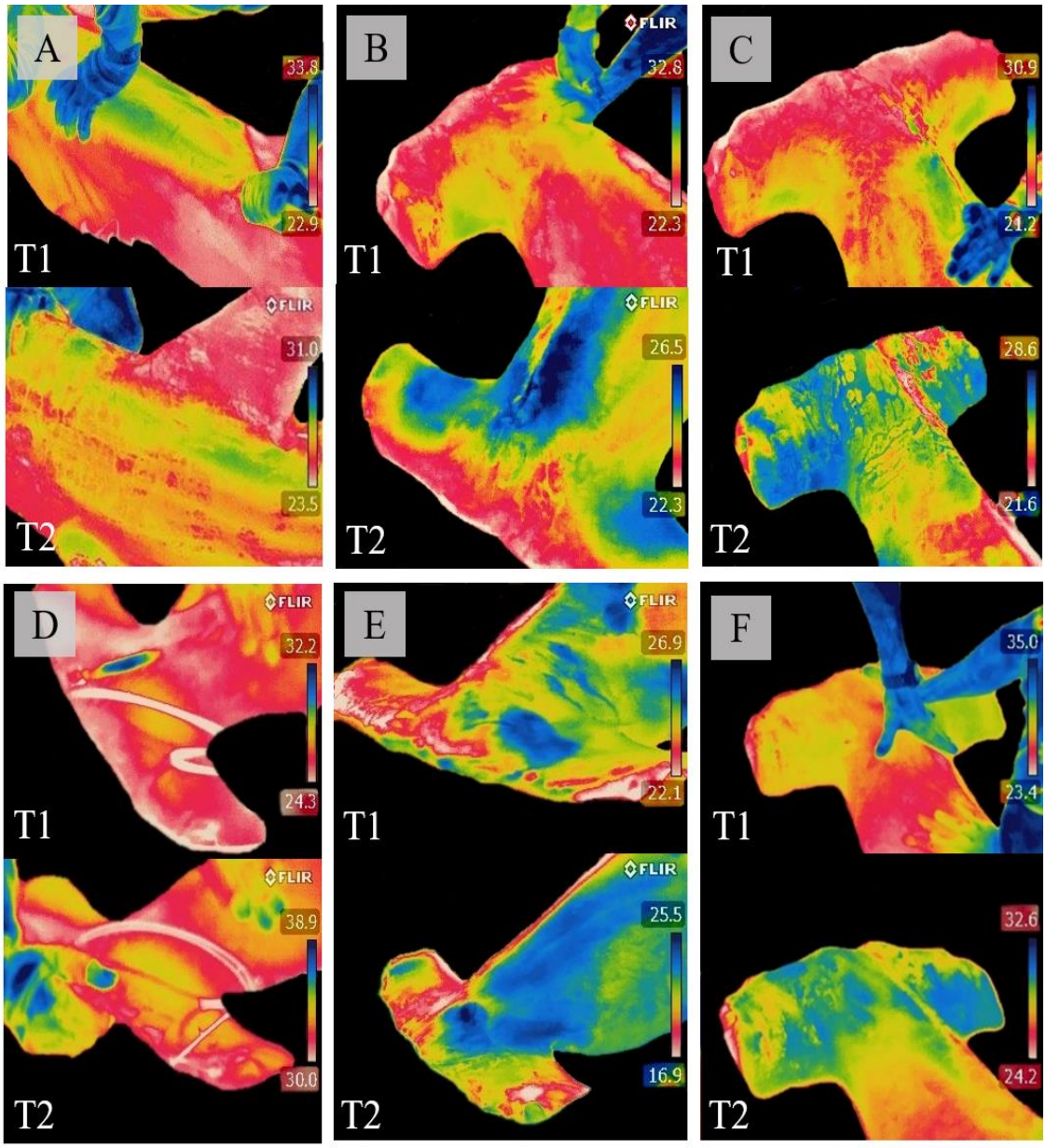



**Figure 1.** Representative infrared images of the body from *S. mokarran* (A) at one minute (T1) and two minutes (T2) of experimental exposure to solar radiation. Infrared images of the cephalofoil from females (B-F) at one minute (T1) and two minutes (T2) of exposure to solar radiation. The thermal scale bar on right shows the temperature gradient.

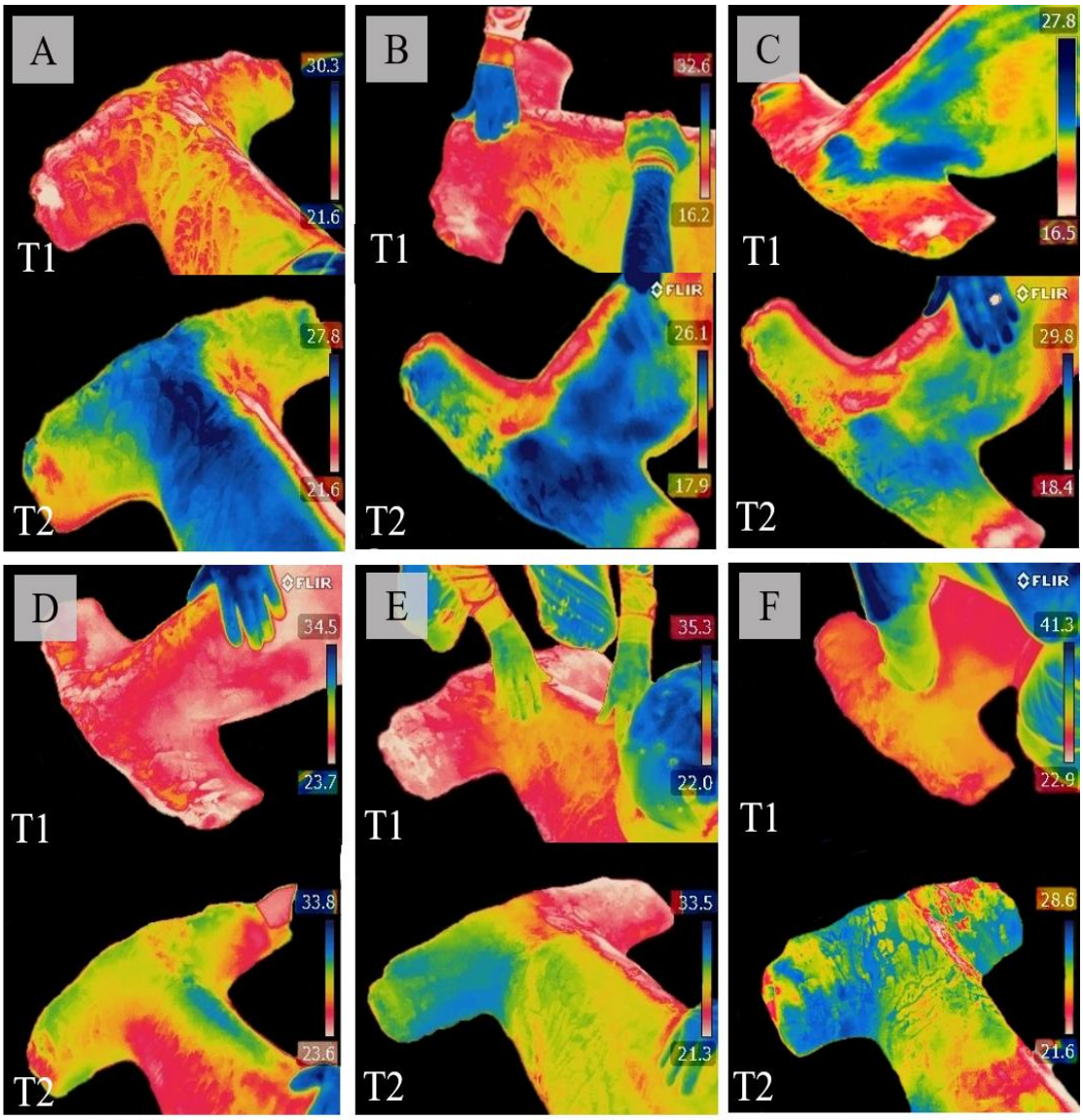



**Figure 2.** Infrared images of the cephalofoil from males (A-F) at one minute (T1) and two minutes (T2) of experimental exposure to solar radiation. The thermal scale bar on right shows the temperature gradient.

The rapid cephalofoil warming of great hammerheads exposed to solar radiation that we documented here likely has a physical (versus metabolic) origin, due to a high cephalofoil surface area to volume ratio. Indeed, there was no statistical difference between the water temperature and the cephalofoil in the first minute after exposure (Student's t-test; t=-1.398, P=0.177), and no statistical difference between air temperature and cephalofoil at the second minute (Student's t-test; t=-0.657 P=0.519) consistent with a strong relationship between increase cephalofoil temperature due to increasing air exposure (solar radiation) (Figure 3).

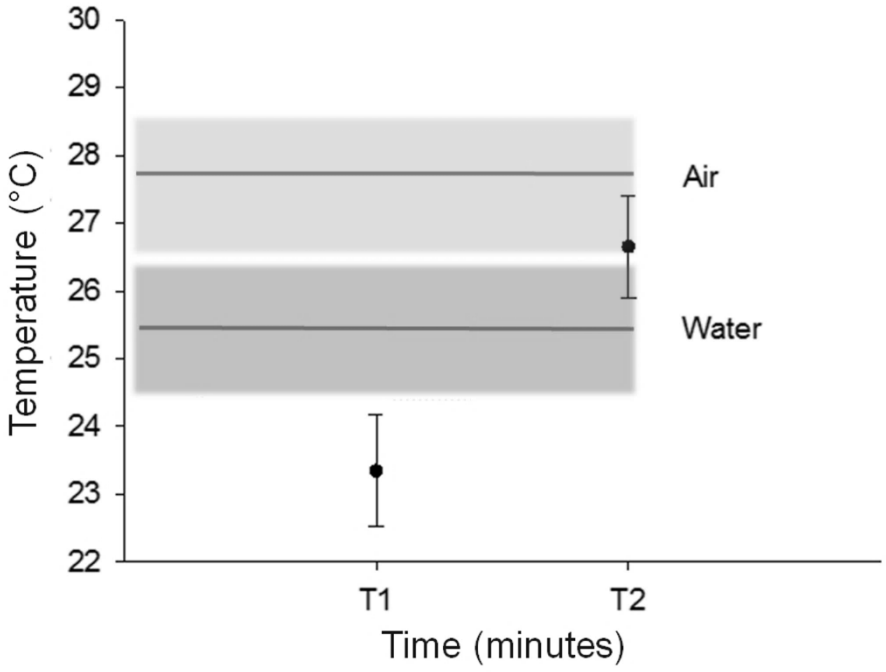



**Figure 3.** Comparison of measured water temperatures at capture versus cephalofoil temperatures at the first minute post exposure to solar radiation (T1) as well as comparisons of measured air temperature versus cephalofoil temperature (T2) at second minute post experimental exposure to solar radiation. Black circles are mean cephalofoil temperatures (°C) ± standard error (wiskers). The further down bold line is the mean water temperature (°C) ± standard error (dark grey shaded area). The upper bold line indicates mean air temperature (°C) ± standard error (light grey shaded area). Student's t-Tests were used to statistically compare water and air temperatures versus cephalofoil temperatures.

A comparable rapid increase was not observed in the head or body flank of other species captured in the same area at the same time, such as blacknose (*Carcharhinus acronotus*), sandbar (*Carcharhinus plumbeus*), nurse (*Ginglymostoma cirratum*) and blacktip (*Carcharhinus limbatus*) sharks (Figure 4) leading us to believe that in fact, the thermal capacity observed may be unique to hammerhead sharks, since a similar pattern of cephalofoil temperature increase was also documented in the Scalloped hammerhead *Sphyrna lewini* (Figure 5).

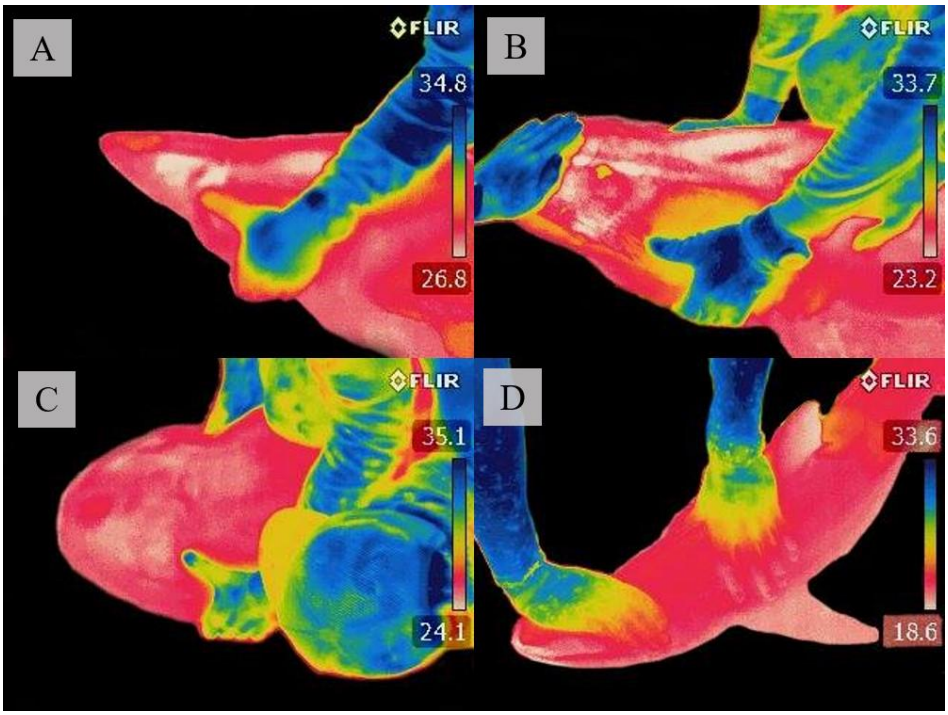



**Figure 4.** Representative infrared thermal images of heads from (A) blacknose (*Carcharhinus acronotus*), (B) sandbar (*Carcharhinus plumbeus*), (C) nurse (*Ginglymostoma cirratum*) and (D) blacktip (*Carcharhinus limbatus*) sharks after two minutes of experimental exposure to solar radiation. The thermal scale bar on right shows the temperature gradient.

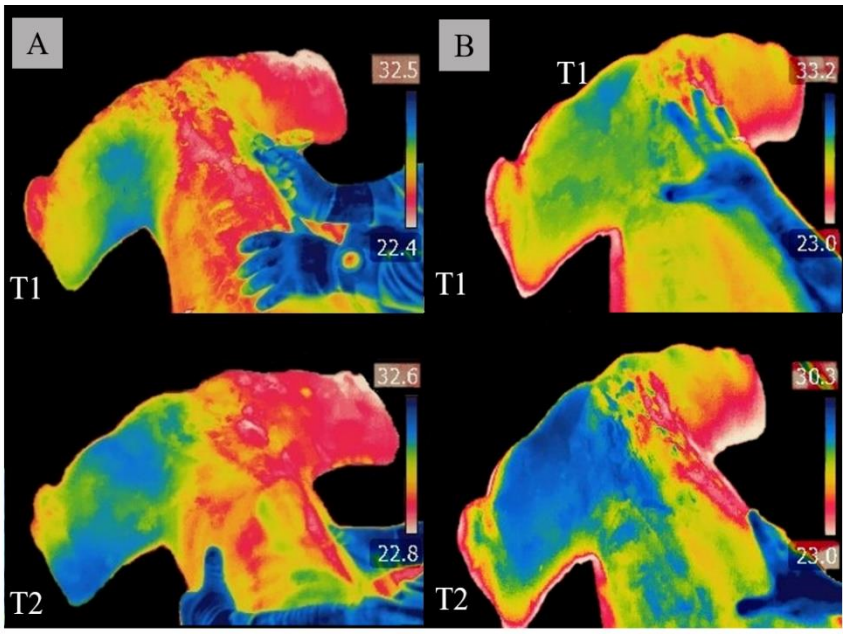



**Figure 5.** Infrared thermal images of the cephalofoil from two Scalloped hammerhead sharks (*S. lewini*) (A-B) after one (T1) and two minutes (T2) of experimental exposure to solar radiation. The thermal scale bar on right shows the temperature gradient.

## 4 Discussion

Given that great hammerhead sharks occupy tropical waters, frequently entering coastal warm waters and have evolved several specializations to occupy and hunt in shallow water under conditions of high solar radiation, including skin color changes (tanning) (Lowe and Goodman-Lowe 1996) and ocular adaptations as a response to brighter environments (Nelson 2003). Therefore, it stands to reason that the measured accommodation in cephalofoil temperature may be an evolutionary specialization exhibited by the family that allows them to effectively occupy shallow environments and rapidly move horizontally or vertically between water of different temperatures. Accordingly, we hypothesize that the proposed thermal accommodation of the cephalofoil enables rapid temperature equilibrium in the electrosensors (which are located near the skin's surface) (Figure 6). As consequence, this feature may protect the shark's electrosensory system from overexcitement, and subsequent reduction in perception ability, due to voltage changes that would otherwise be driven by increased water temperatures as detailed above.

Sharks exhibit a highly developed electrosensory system composed by groups of subdermal ampullae that are capable of detecting electric fields less than 5 nV cm-1 (Kalmijn 1971). Each ampullae consists of a small chamber and a 1 mm subdermal channel filled with an ion-rich jelly that juts toward the skin surface (Waltman 1966). Besides the ability to detect electrical and magnetic fields, the ampullae have shown themselves to be incredibly sensitive to temperature changes and gradients (Akoev *et al.* 1980; Wissing *et al.* 1988). An inhomogeneous temperature in an ampulla and its canal would lead to a voltage signal, triggering substantial (presumably disadvantageous) responses in the electrosensory system (Brown 2003, 2010), such would occur if a shark were to move rapidly between cold and warm water as these animals do.

Electrophysiology trials of excised ampullae have demonstrated that sudden temperature gradients of less than 1˚C lead to 300% increases in associated primary afferent firing rate (Wissing *et al.* 1988). The effect is transient as long as thermal equilibrium (equal temperatures throughout the system) is achieved. According to measurements of the thermoelectric character of the ampullary gel, an ampulla-canal system encountering a full 1˚C temperature

inhomogeneity would encounter an electrical signal spike of 200-300 μV, presumably overwhelming the electrosensors, which have shown sensitivity levels as low as 1 nV (Kalmijn 1971; Brown 2010). As a shark swims into warmer shallow waters, temperature gradients of at least 1˚C will be commonplace and – without rapid thermal accommodation leading to more homogeneous temperatures in the electrosensory system – an otherwise inhomogeneous temperature in an ampulla and its canal would lead to over-stimulation of the electrosensory system (Brown 2003, 2010). In order to avoid over-stimulation via such thermo-electric signals, the electrosensors need to preserve a relatively stable temperature in the canals and ampullae. If the cephalofoil morphology allows for rapid temperature equilibrium in the electrosensors after entering a new ambient water temperature, it would indeed be advantageous. Future studies combining both infrared technology and electroencephalography may provide insights into our hypothesis.


**Figure 6.** Thermal accommodation theory. The scheme exhibit the increase in the cephalofoil temperature as an evolutionary ability to properly accommodate the ampullae to an abrupt change in the surrounding temperature when entering in shallow waters, ensuring this way a proper electrical perception during foraging. Scheme by National Geographic on Mega Hammerhead special (SharkFest 2016).

*Sphyrna mokarran* features one of the largest cephalofoils and telencephalons amongst sharks, a fact that, combined with possible cephalofoil

thermal accommodation hypothesized here, may aid electrosensory activity in warmer environments. The ability to warm the brain and eyes to increase cognitive ability and visual acuity is reported for endothermic sharks (Block and Carey 1985) and swordfishes (Carey 1982). It is plausible that the observed rapid cephalofoil temperature increase could also increase brain and eye temperatures which could improve spatial awareness and visual acuity when hunting. These factors may also play a role in the performance of complex social behaviors and hunting strategies known for this species (Gallagher *et al.* 2014a).

Several studies have shown a strong correlation between air temperature and solar radiation (Presscott and Collins, 1951; Walter 1969; Budyko 1969; Chang and Root 1975; Bristow and Campbell 1984; Allen 1995; Prieto *et al.* 2009) leading us to suspect that the rapid increases in cephalofoil temperatures that we measured during experimental air exposure is due to heat absorption from solar radiation. It is obvious that associated changes in the cephalofoil temperature would be lower underwater, due to the high thermal conductivity of water, combined with lower penetration of solar radiation. However, thermal accommodation, possibly triggered by absorption of heat from solar radiation would still be possible, as these animals tend to swim near the surface, where vertical transmittance ($E_{SR}$) is still high (Lee *et al.* 2005; Lin *et al.* 2016).

If the thermal accommodation proposed here is driven by solar radiation and closely related to warmer environment use, it is plausible to infer that climate change may affect this feature. Intergovernmental Panel on Climate Change has predicted an increase of 1°C in waters of the Atlantic Ocean under RCP2.6 (Representative Concentration Pathways – radiative forcing of 2.6 W/m$^2$) and an increase of 3-5°C under RCP8.5 (Representative Concentration Pathways – radiative forcing of 8.5 W/m$^2$) (IPCC 2014). In contrast, solar radiation is predicted to become reduced on earth's penetration and retention, manly related to the ozone layer recovery (Bais *et al.* 2011). The geographic range of great hammerhead sharks is primarily within latitudes 40° N to 35° S (Compagno 2001). This range is predicted to experience large increases in water temperatures (~14%) (IPCC 2014). However, within the same range, solar radiation is predicted to reduce (3.3-4.1%) or a slight increase (0.8-1.1%) (Bais *et al.* 2011) (Figure 7). Thus, if great hammerheads are indeed able to benefit from solar radiation

permitting rapid warming of the cephalofoil that consequently prevents overexcitement of the electrosensory system when entering warmer water (i.e. thermal accommodation hypothesized here), then under future climate change scenarios this feature may become compromised due to large increases in water temperature without concurrent increases in solar radiation.

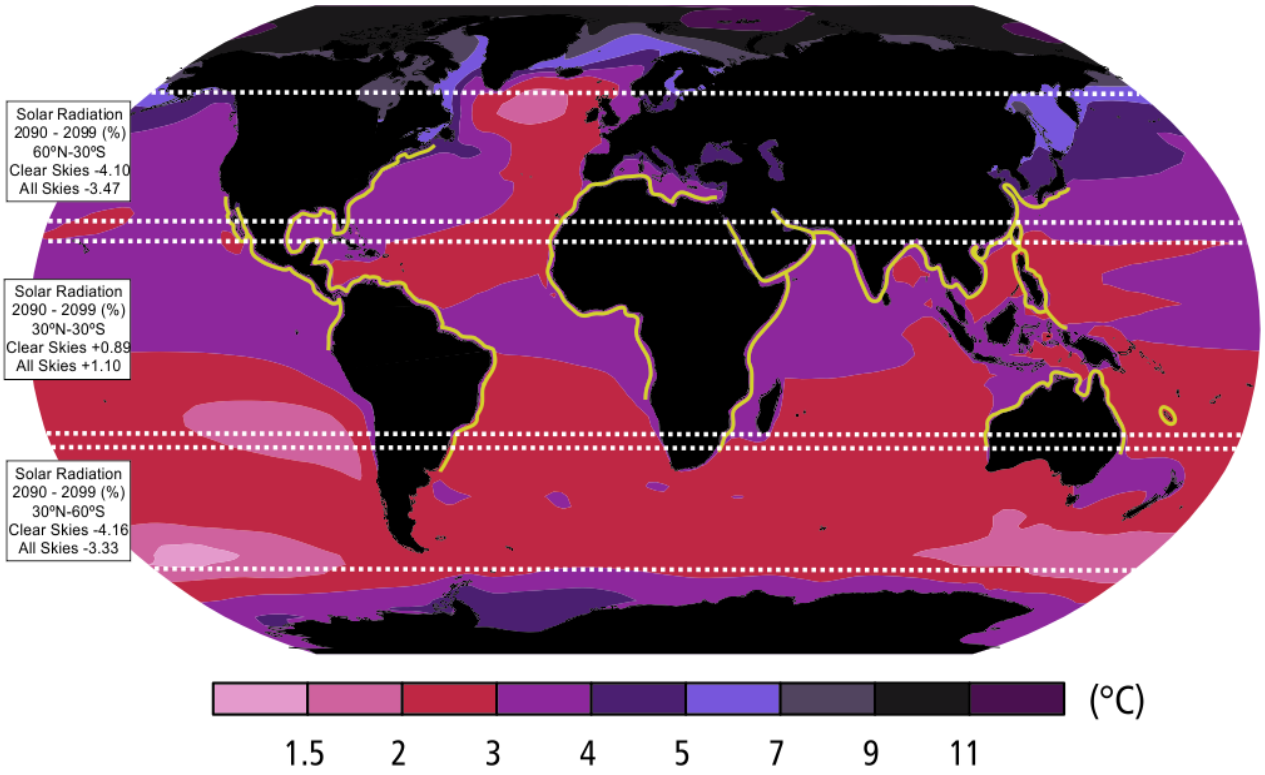



**Figure 7.** Geographical comparison of *S. mokarran* distribution (based on Compagno 2001) versus both sea surface temperatures and solar radiation predictions for 2100, for RCP8.5 (IPCC 2014) and SRES A1B (Bais *et al.* 2010) scenarios, respectively. The current distribution of *S. mokarran* is identified by dark red contours along coastal shorelines, primarily bound between latitudes 45°N and 35°S. With parallel lines enclose latitudinal areas that will experience predicted reductions in solar radiation in year 2100. The related solar radiation values are provided in the white boxes bound by the associated latitudinal bounding areas demarked by withe parallel lines. Figure by Renata D. Leite.

## 5 Conclusion

In summary, we used thermal imaging to document rapid (< 2 minutes) temperature increases (3.1± 0.56˚C per-minute) in the cephalofoil of great hammerheads experimentally exposed to direct solar radiation that we hypothesize is an evolutionary specialization allowing this species to rapidly enter

and exploit warm surface or shallow waters (i.e., thermal accommodation capacity). It has recently been argued that the novel specializations that have historically contributed to evolutionary success in hammerheads have become maladaptive under current conditions due to human's stressors, leading to high risk of extinction. Based on the data reported here and possible underlying mechanism, we hypothesize that the thermal accommodation capacity in great hammerheads proposed here may become disrupted due to predicted changes in water temperature and solar radiation under future climate change scenarios.

## Literature cited

Allen, R. G. (1995). Evaluation of Procedures for Estimating Mean Monthly Solar Radiation from Air Temperature, Technical Report, United Nations Food and Agric. Org. (FAO), Rome, Italy.

Akoev, G. N., Volpe, N. O., Zhadan, G. G. (1980). Analysis of effects of chemical and thermal stimuli on the ampullae of Lorenzini of the skates. Comparative Biochemistry and Physiology (A), 65, 193-201.

Bais, A. F., Tourpali, K. A. Kazantzidis, A., Akiyoshi, H., Bekki, S., Braesicke, P., et al. (2011). Projections of UV radiation changes in the 21st century: impact of ozone recovery and cloud effects. Atmospheric Chemistry and Physics, 11, 7533–7545.

Bennett, A. F., Seymour, R. S., Bradford, D. F., Webb, G. J. W. (1985). Mass-dependence of anaerobic metabolism and acid-base disturbance during activity in the salt-water crocodile, *Crocodylus porosus*. Journal of Experimental Biology, 118, 161-171.

Bristow, K. L., Campbell, G. S. (1984). On the relationship between incoming solar radiation and daily maximum and minimum temperature. Agricultural and Forest Meteorology, 31, 159–166.

Brown, B. R. (2003). Neurophysiology: sensing temperature without ion channels. Nature, 421, 495-495.

Brown, B. R. (2010). Temperature response in electrosensors and thermal voltages in electrolytes. Journal of Biological Physics, 36, 121-134.

Block, B. A., Carey, F. G. (1985). Warm brain and eye temperatures in sharks. Journal of Comparative Physiology (B), 156, 229-236.

Budyko, M. I. (1969). The Effect of Solar Radiation Variations on the Climate of the Earth. Tellus, 21, 611-619.

Carey, F. G. (1982). A brain heater in the swordfish. Science, 216, 1327-1329.

Chang, J. H., Root, B. (1975). On the relationship between mean monthly global radiation and air temperature. Archiv für Meteorologie, Geophysik und Bioklimatologie (B), 23(1-2), 13-30.

Chapman, D. D., Gruber, S. H. (2002). A further observation of the prey-handling behavior of the great hammerhead shark, *Sphyrna mokarran*: Predation upon the spotted eagle ray, *Aetobatus narinari*. Bulletin of Marine Science, 70, 947-952.

Compagno, L. J. V. (2001). Sharks of The World: An Annotated and Illustrated Catalogue of Shark Species Known to Date. Vol. 2. No. 1. Food Agriculture Org.

Gallagher, A. J., Hammerschlag, N., Shiffman, D. S., Giery, S. T. (2014a). Evolved for extinction: the cost and conservation implications of specialization in hammerhead sharks. BioScience, 64, 619-624.

Gallagher, A. J., Serafy, J. E., Cooke, S. J., Hammerschlag, N. (2014b). Physiological stress response, reflex impairment, and survival of five sympatric shark species following experimental capture and release. Marine Ecology Progress Series, 496, 207-218.

Graham, F., Rynne, P., Estevanez, M., Luo, J., Ault, J. S., Hammerschlag, N. (2016). Use of marine protected areas and exclusive economic zones in the subtropical western North Atlantic Ocean by large highly mobile sharks. Diversity and Distributions, 1-13.

IPCC Fifth Assessment Report - Impacts, Adaptation and Vulnerability. 2014. Available: https://www.ipcc.ch/publications_and_data/ar4/wg1/en/spmsspm-projections-of.html

Kajiura, S. M., Forni, J. B., Summers AP. (2003). Maneuvering in juvenile carcharhinid and sphyrnid sharks: the role of the hammerhead shark cephalofoil. Zoology, 106, 19-28.

Kalmijn, A. J. (1971). The electric sense of sharks and rays. Journal of Experimental Biology, 71, 55 371-383.

Lee, Z., Du, K., Arnone, R., Liew, S., Penta, B. (2005). Penetration of solar radiation in the upper ocean: A numerical model for oceanic and coastal waters. Journal of Geophysical Research: Oceans, 110(C9).

Lim, D. D., Motta, P., Mara, K., Martin, A. P. (2010). Phylogeny of hammerhead sharks (Family Sphyrnidae) inferred from mitochondrial and nuclear genes. Molecular Phylogenetics and Evolution, 55(2), 572-579.

Lin, J., Lee, Z., Ondrusek, M., Kahru, M. (2016). Attenuation coefficient of usable solar radiation of the global oceans. Journal of Geophysical Research: Oceans.

Lowe, C., Goodman-Lowe, G. (1996). Sun tanning in hammerhead sharks. Nature, 383, 677.

McComb, D. M., Tricas, T. C., Kajiura, S. M. (2009). Enhanced visual fields in hammerhead sharks. Journal of Experimental Biology, 212, 4010-4018.

Nakaya, K. (1999). Hydrodynamic function of the head in the hammerhead sharks (Elasmobranchii: Sphyrnidae). Copeia, 330-336.

Naylor, G. J. P. (1992). The phylogenetic relationships among requiem and hammerhead sharks: inferring phylogeny when thousands of equally most parsimonious trees result. Cladistics, 8, 295-318.

Nelson, P., Kajiura, S. M., Losey, G. (2003). Exposure to solar radiation may increase ocular UV-filtering in the juvenile scalloped hammerhead shark, *Sphyrna lewini*. Marine Biology, 142, 53-56.

Presscott, J. A., Collins, J. C. (1951). The Lag of Temperature Behind Solar Radiation. Quarterly Journal of the Royal Meteorological Society, 52, 121-126.

Prieto, J. I., Martínez-García, J. C., García, D. (2009). Correlation between global solar irradiation and air temperature in Asturias, Spain. Solar Energy, 83(7), 1076-1085.

Roemer, R. P., Gallagher, A. J., Hammerschlag, N. (2016). Shallow water tidal flat use and associated specialized foraging behavior the great hammerhead

shark (*Sphyrna mokarran*). Marine and Freshwater Behavior and Physiology, 1-15.

Schaller, G. B. (1968). Hunting behavior of the cheetah in the Serengeti National Park, Tanzania. African Journal of Ecology, 6 95-100.

Tester, A. L. Olfaction, gustation, and the common chemical sense in sharks. (1983). Sharks and Survival, 8, 255-282.

Tricas, T. C., Kajiura, S. M., Summers, A. P. (2009). Response of the hammerhead shark olfactory epithelium to amino acid stimuli. Journal of Comparative Physiology (A), 195, 947-954.

Yopak, K. E., Lisney, T. J., Collin, S. P., Montgomery, J. C. (2007). Variation in brain organization and cerebellar foliation in chondrichthyans: sharks and holocephalans. Brain, Behavior and Evolution, 69, 280-300.

Yopak, K. E., Balls, G., Frank, L. (2009). Cortical surface structure analysis in sharks using magnetic resonance imaging (MRI). Proc ISMRM, 17, 2925.

Yopak, K. E. (2012). Neuroecology of cartilaginous fishes: the functional implications of brain scaling. Journal of Fish Biology, 80, 1968-2023.

Walter, A. A. (1969). Relationship between Incoming Solar Radiation and Degree hours of Temperature. Agricultural Meteorology, 6, 435.

Waltman, B. (1966). Electrical properties and fine structure of the ampullary canals of Lorenzini. Acta Physiologica Scandinavica. Supplementum Journal, 264, 1.

Weber, K., Sturmer, L., Hoover, E., Baker, S. (2007). The role of water temperature in hard clam aquaculture. UF/IAS Extension. Fisheries and Aquatic Science Department.

Williams, S. R., Stuarty, G. J. (1999). Mechanisms and consequences of action potential burst firing in rat neocortical pyramidal neurons. Journal of Physiology, 521, 467-482.

Wissing, H., Braun, H. A., Schafer, K. (1988). Dynamic response characteristics of the ampullae of Lorenzini to thermal and electrical stimuli. Progress in Brain Research, 74, 99-107.

## GENERAL CONCLUSION

This thesis had as main objective to establish physiology as an efficient tool for conservation, based on fishing and climatic change pressures in elasmobranchs. Despite being a traditional line of research, physiology is rarely used in an applied and conservation-oriented way. The results obtained in chapter 1 demonstrate the reduction of post-capture recovery ability in females during the gestation period, bringing important data to the improvement of management plans based on compensatory release of this threatened species with low commercial value commonly landed still alive by artisanal fishing. Chapter 2 brings the validation of a new approach to traditional stress studies in sharks. Since it is virtually impossible to collect baseline data, this chapter aimed to test the use of a negative control so that a comparative database can be established, being possible to determine which levels of stress response are close to the lethality. Chapters 3 and 4 discuss the effects of air exposure in a new approach: analyzing the effects of air temperature and solar radiation on the body temperature and how such alteration may affect the survival capacity of the species tested. The results provide important points that should be taken into account to the improvement of recreational fisheries legislation, which currently does not determine the maximum time a fish caught can be out of water. Finally, chapters 5 and 6 aimed to analyze the effects of thermal and saline changes on two endangered species. The results show that both present difficulties in dealing with abiotic changes, bringing important points that should be better discussed in an ecophysiological and conservationist context. Among the results obtained, it was possible to elucidate some of the mechanisms activated by stress, thus bringing important data to be considered in the management plans of the species studied here.

# GENERAL REFERENCES


Abilhoa, V., Bornatowski, H. Freitas, M.O. (2007). Some information on reproduction and embryonic development of the lesser guitarfish *Zapteryx brevirostris* in Southern Brazil. Acta Adriatica, 48, 89–94.

Afonso, A. S., Hazin, F. H., Carvalho, F., Pacheco, J. C., Hazin, H., Kerstetter, D. W., ... Burgess, G. H. (2011). Fishing gear modifications to reduce elasmobranch mortality in pelagic and bottom longline fisheries off Northeast Brazil. Fisheries Research, 108(2), 336-343.

Akoev, G. N., Volpe, N. O., Zhadan, G. G. (1980). Analysis of effects of chemical and thermal stimuli on the ampullae of Lorenzini of the skates. Comparative Biochemistry and Physiology (A), 65, 193-201.

Albers, C. (1970). Acid-base balance. In: Fish Physiology. Hoar W, Randall DJ (eds). Vol. IV. Academic Press Inc., United States pp.173-205.

Allen, R. G. (1995). Evaluation of Procedures for Estimating Mean Monthly Solar Radiation from Air Temperature, Technical Report, United Nations Food and Agric. Org. (FAO), Rome, Italy.

Anderson, W. G., Takei, Y., Hazon, N. (2002). Osmotic and volaemic effects on drinking rate in elasmobranch fish. Journal of experimental biology, 205(8), 1115-1122.

Anderson, W. G. (2012). The endocrinology of 1α-hydroxycorticosterone in elasmobranch fish: A review. Comparative Biochemistry and Physiology (A), 162(2),73-80.

Andrigueto, J. M., Peryl, L., Minardi, I. (1990). Nutrição animal: bases e fundamentos da nutrição animal. Vol. I. Nobel 1, Brazil 396pp.

Arlinghaus, R., Klefoth, T., Kobler, A., Cooke, S. J. (2008). Size selectivity, injury, handling time, and determinants of initial hooking mortality in recreational angling for northern pike: the influence of type and size of bait. North American Journal of Fisheries Management, 28(1), 123-134.

Armour, K. J., O'Toole, L. B., Hazon, N. (1993). The effect of dietary protein restriction on the secretory dynamics of 1α-hydroxycorticosterone and urea in the dogfish, *Scyliorhinus canicula*: a possible role for 1α-hydroxycorticosterone in sodium retention. Journal of Endocrinology, 138(2), 275-282.

Awruch, C. A., Simpfendorfer, C., Pankhurst, N. W. (2011). Evaluation and use of a portable field kit for measuring whole-blood lactate in sharks. Marine and Freshwater Research, 62, 694-699.

Awruch, C. A. (2013). Reproductive endocrinology in chondrichthyans: the present and the future. General and comparative endocrinology, 192, 60-70.

Bais, A. F., Tourpali, K. A. Kazantzidis, A., Akiyoshi, H., Bekki, S., Braesicke, P., et al. (2011). Projections of UV radiation changes in the 21st century: impact of ozone recovery and cloud effects. Atmospheric Chemistry and Physics, 11, 7533–7545.

Baldwin, J., Wells, R. M. G. (1990). Oxygen transport potential in tropical elasmobranchs from the Great Barrier Reef: relationship between hematology and blood viscosity. Journal of Experimental Marine Biology and Ecology, 144(2), 145-155.

Ballantyne, J. S., Robinson, J. W. (2010). Freshwater elasmobranchs: a review of their physiology and biochemistry. Journal of Comparative Physiology B, 180(4), 475-493.

Ballantyne, J. S. (2015). Jaws II: Metabolism of Elasmobranchs. In: Physiology of Elasmobranch Fishes: Internal Processes. Shadwick RE, Farrell AP, Brauner CJ (eds). 1st edition. Academic Press, United States, pp. 395-456.

Ballantyne, J. S. (2016). Some of the most interesting things we know, and don't know, about the biochemistry and physiology of elasmobranch fishes (sharks, skates and rays). Comparative Biochemistry and Physiology (B).

Balmaceda-Aguilera, C., Cortes-Campos, C., Cifuentes, M., Peruzzo, B., Mack, L., Tapia, J. C., et al. (2012). Glucose transporter 1 and monocarboxylate transporters 1, 2, and 4 localization within the glial cells of shark blood-brain-barriers. PloS one 7(2):e32409.

Barker, M. J., Schluessel, V. (2005). Managing global shark fisheries: suggestions for prioritizing management strategies. Aquatic Conservation, 15, 325-347.

Barton, B. A., Iwama, G. K. (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Review of Fish Diseases, 1, 3-26.

Batista, V. S. (1991). Aspectos quantitativos da fecundidade e do desenvolvimento embrionário da raia *Zapteryx brevirostris* Müller and Henle 1841 (Pisces, Rhinobatidae) da Enseada de Itaipu, Niterói, Rio de Janeiro. Revista Brasileira de Biologia, 51(3), 495-501.

Becker, C. D., Genoway, R. G. (1979). Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. Environmental Biology of Fishes, 4(3), 245-256.

Beerkircher, L. R., Cortes, E., Shivji, M. (2002). Characteristics of shark's bycatch observed on pelagic loglines off Southeastern United States, 1992-2000. Marine Fisheries Review, 64(4), 40-49.

Bennett, A. F., Seymour, R. S., Bradford, D. F., Webb, G. J. W. (1985). Mass-dependence of anaerobic metabolism and acid-base disturbance during activity in the salt-water crocodile, *Crocodylus porosus*. Journal of Experimental Biology, 118, 161-171.

Block, B. A., Carey, F. G. (1985). Warm brain and eye temperatures in sharks. Journal of Comparative Physiology (B), 156, 229-236.

Bonga, S. W. (1997). The stress response in fish. Physiological Reviews, 77(3), 591-625.

Bone, Q. (1988). Muscles and locomotion. In: Physiology of elasmobranch fishes. Shuttleworth TJ (editor). Springer-Verlag, Berlin, pp.99–141.

Boonstra, R. (2004). Coping with changing northern environments: the role of the stress axis in birds and mammals. Integrative and Comparative Biology, 44(2), 95-108.

Bornatowski, H., Abilhoa, V. (2012). Tubarões e raias capturados pela pesca artesanal no Paraná: guia de identificação. Hori Cadernos Técnicos, (4).

Bornatowski, H., Braga, R. R., Vitule, J. R. S. (2013). Shark mislabeling threatens biodiversity. Science, 340(6135), 923-923.

Braccini, M., McAuley, R., Rowland, F. (2013). Temperate demersal gillnet and demersal longline fisheries status report, p. 271–284. In: Status reports of the fisheries and aquatic resources of Western Australia 2012/13: The state of fisheries. W. J. Fletcher, and K. Santoro (eds). Department of Fisheries, Western Australia.

Braithwaite, V. A., Ebbesson, L. O. E. (2014). Pain and stress responses in farmed fish. Rev Sci Tech, 33(1), 245-253.

Brajon, S., Laforest, J. P., Schmitt, O., Devillers, N. (2016). A preliminary study of the effects of individual response to challenge tests and stress induced by humans on learning performance of weaned piglets (Sus scrofa). Behavioral Process, 129, 27-36.

Brill, R., Bushnell, P., Schroff, S., Seifert, R., Galvin, M. (2008). Effects of anaerobic exercise accompanying catch-and-release fishing on blood-oxygen affinity of the sandbar shark (*Carcharhinus plumbeus*, Nardo). Journal of Experimental Marine Biology and Ecology, 354(1), 132-143.

Bristow, K. L., Campbell, G. S. (1984). On the relationship between incoming solar radiation and daily maximum and minimum temperature. Agricultural and Forest Meteorology, 31, 159–166.

Brooks, T. M., Mittermeier, R. A., da Fonseca, G. A., Gerlach, J., Hoffmann, M., Lamoreux, J. F., ... Rodrigues, A. S. (2006). Global biodiversity conservation priorities. Science, 313(5783), 58-61.

Brooks, E. J., Sloman, K. A., Liss, S., Hassan-Hassanein, L., Danylchuk, A. J., Cooke, S. J., Mandelman, J. W., Skomal, G. B., Sims, D. W., Suski, C. D. (2011). The stress physiology of extended duration tonic immobility in the juvenile lemon shark, *Negaprion brevirostris* (Poey 1868). Journal of Experimental Marine Biology and Ecology, 409, 351–360.

Brooks, E. J., Mandelman, J. W., Sloman, K. A., Liss, S., Danylchuk, A. J., Cooke, S. J., Skomal, G. B., Philipp, D. P., Sims, D. W., Suski, C. D. (2012). The physiological response of the Caribbean reef shark (*Carcharhinus perezi*) to longline capture. Comparative Biochemistry and Physiology (A), 162, 94–100.

Brown, B. R. (2003). Neurophysiology: sensing temperature without ion channels. Nature, 421, 495-495.

Brown, B. R. (2010). Temperature response in electrosensors and thermal voltages in electrolytes. Journal of Biological Physics, 36, 121-134.

Brown, L. R., Komoroske, L. M., Wagner, R. W., Morgan-King, T., May, J. T., Connon, R. E., Fangue, N. A. (2016). Coupled Downscaled Climate Models and Ecophysiological Metrics Forecast Habitat Compression for an Endangered Estuarine Fish. PloS one, 11(1), e0146724.

Budyko, M. I. (1969). The Effect of Solar Radiation Variations on the Climate of the Earth. Tellus, 21, 611-619.

Burger, J. W. (1965). Roles of the rectal gland and the kidneys in salt and water excretion in the spiny dogfish. Physiological Zoology, 38, 191–196.

Calosi, P., Bilton, D. T., Spicer, J. I. (2008). Thermal tolerance, acclimatory capacity and vulnerability to global climate change. Biology Letters, 4(1), 99-102.

Cannon, W. B. (1929). Organization for physiological homeostasis. Physiological Reviews, 400, 399-431

Carey, F. G. (1982). A brain heater in the swordfish. Science, 216, 1327-1329.

Carlson, J. K., Goldman, K. J., Lowe, C. G., (2004). Metabolism, energetic demand, and endothermy. In: Biology of Sharks and Their Relatives. Carrier, J.C., Musick, J.A., Heithaus, M.C. (eds) CRC Press LLC, Boca Raton, FL, pp. 203–224.

Castillo-Páez, A., Sandoval-Castillo, J., Corro-Espinosa, D., Tovar-Ávila, J., Blanco-Parra, M. D. P., Saavedra-Sotelo, N. C., ... & Rocha-Olivares, A. (2017). Cutting through the Gordian knot: unravelling morphological, molecular, and biogeographical patterns in the genus Zapteryx (guitarfish) from the Mexican Pacific. ICES Journal of Marine Science, fsx021.

Chang, J. H., Root, B. (1975). On the relationship between mean monthly global radiation and air temperature. Archiv für Meteorologie, Geophysik und Bioklimatologie (B), 23(1-2), 13-30.

Chapman, D. D., Gruber, S. H. (2002). A further observation of the prey-handling behavior of the great hammerhead shark, *Sphyrna mokarran*: Predation upon the spotted eagle ray, *Aetobatus narinari*. Bulletin of Marine Science, 70, 947-952.

Cherry, D. S., Dickson, K. L., Cairns Jr, J., Stauffer, J. R. (1977). Preferred, avoided, and lethal temperatures of fish during rising temperature conditions. Journal of the Fisheries Board of Canada, 34(2), 239-246.

Cheung, W. W., Lam, V. W., Sarmiento, J. L., Kearney, K., Watson, R., Pauly, D. (2009). Projecting global marine biodiversity impacts under climate change scenarios. Fish and Fisheries, 10(3), 235-251.

Chin, A., Kyne, P. M., Walker, T. I., McAuley, R. O. R. Y. (2010). An integrated risk assessment for climate change: analyzing the vulnerability of sharks and rays on Australia's Great Barrier Reef. Global Change Biology, 16(7), 1936-1953.

Chopin, F. S. Arimoto, T. (1995). The condition of fish escaping from fishing gears – a review. Fisheries Research 21, 315–327.

Cicia, A. M., Schlenker, L. S., Sulikowski, J. A. Mandelman, J.W. (2012). Seasonal variations in the physiological stress response to discrete bouts of aerial exposure in the little skate, *Leucoraja erinacea*. Comparative Biochemistry and Physiology A 162, 130–138.

Cilulko, J., Janiszewski, P., Bogdaszewski, M., Szczygielska, E. (2013). Infrared thermal imaging in studies of wild animals. European Journal of Wildlife Research, 59(1), 17-23.

Clark, J. R., Bragin, G. E., Febbo, E. J., Letinski, D. J. (2001). Toxicity of physically and chemically dispersed oils under continuous and environmentally realistic exposure conditions: Applicability

to dispersant use decisions in spill response planning. In International Oil Spill Conference, 2, 1249-1255. American Petroleum Institute.

Clarke, S. C., McAllister, M. K., Milner-Gulland, E. J., Kirkwood, G. P., Michielsens, C. G., Agnew, D. J., ... Shivji, M. S. (2006a). Global estimates of shark catches using trade records from commercial markets. Ecology letters, 9(10), 1115-1126.

Clarke, S. C., Magnussen, J. E., Abercrombie, D. L., McAllister, M. K., Shivji, M. S. (2006b). Identification of shark species composition and proportion in the Hong Kong shark fin market based on molecular genetics and trade records. Conservation Biology, 20(1), 201-211.

Cliff, G., Thurman, G. D. (1984). Pathological and physiological effects of stress during capture and transport in the juvenile dusky shark, *Carcharhinus obscurus*. Comparative Biochemistry and Physiology (A), 78,167–173.

Cockrem, J. F. (2013). Individual variation in glucocorticoid stress responses in animals. General and Comparative Endocrinology, 181, 45-58.

Compagno, L. J. V. (2001). Sharks of The World: An Annotated and Illustrated Catalogue of Shark Species Known to Date. Vol. 2. No. 1. Food Agriculture Org.

Conrath, C.L. (2004). Reproductive biology. In: Elasmobranch fisheries management techniques. Musick JA, Benfil R (eds) APEC Fisheries Working Group, pp. 133–164.

Cooke, S. J., Schreer, J. F., Wahl, D. H., Philipp, D. P. (2000). Locomotory dynamics as indicators of stress in fish—remote measures utilizing activity transmitters. In Stress in Fish: New Directions. Proceedings of the International Congress on the Biology of Fish, University of Aberdeen, Scotland, 77-86.

Cooke, S.J., Suski, C.D., Barthel, B.L., Ostrand, K.G., Tufts, B.L., Philipp, D.P., (2003). Injury and mortality induced by four hook types on blue gill and pumpkin seed. North American Journal of Fisheries Management, 23, 883–893.

Cooke, S. J., Sack, L., Franklin, C. E., Farrell, A. P., Beardall, J., Wikelski, M., Chown, S. L. (2013). What is conservation physiology? Perspectives on an increasingly integrated and essential science†. Conservation Physiology, 1(1), cot001.

Cooper, A. R., Morris, S. (1998). The blood respiratory, haematological, acid–base and ionic status of Port Jackson shark, *Heterodontus portusjacksoni*, during recovery from anesthesia and surgery: a comparison with sampling by direct caudal puncture. Comparative Biochemistry and Physiology (A), 119A, 895–903.

Costa, L., Chaves, P. D. T. D. C. (2006). Elasmobrânquios capturados pela pesca artesanal na costa sul do Paraná e norte de Santa Catarina, Brasil. Biota Neotropica, 6(3), 1-10.

Cuyler, L. C., Wiulsrød, R., Øritsland, N. A. (1992). Thermal infrared radiation from free living whales. Marine Mammal Science, 8(2), 120-134.

Crowder, L. B., Murawski, S. A. (1998). Fisheries bycatch: implications for management. Fisheries, 23(6), 8-17.

Davies, R. W. D., S. J. Cripps, A. Nickson, G. Porter. (2009). Defining and estimating global marine fisheries bycatch. Marine Policy, 33, 661-72.

Davis, M.W. (2002). Key principles for understanding fish by-catch discard mortality. Canadian Journal of Fisheries and Aquatic Sciences 59, 1834–1843.

Davis, M. W., Parker, S. J. (2004). Fish size and exposure to air: potential effects on behavioral impairment and mortality rates in discarded sablefish. North American Journal of Fisheries Management, 24(2), 518-524.

Deck, C. A., LeMoine, C. M. R., Walsh, P. J. (2016). Phylogenetic analysis and tissue distribution of elasmobranch glucose transporters and their response to feeding. Biology Open, 0, 1-6.

Dent, F., Clarke, S. (2015). State of the global market for shark products. FAO Fisheries and Aquaculture Technical Paper, 590.

deRoos, R., deRoos, C. C. (1978). Elevation of plasma glucose levels by catecholamines in elasmobranch fish. General and Comparative Endocrinology, 34, 447–452.

deRoos, R., deRoos, C. C., Werner, C. S., Werner, H. (1985). Plasma levels of glucose, alanine, lactate, and β-hydroxybutyrate in the unfed spiny dogfish (*Squalus acanthias*) after surgery and following mammalian insulin infusion. General and Comparative Endocrinology, 58, 28–43.

Deslauriers, D., Heironimus, L., Chipps, S. R. (2016). Lethal Thermal Maxima for Age-0 Pallid and Shovelnose Sturgeon: Implications for Shallow Water Habitat Restoration. River Research and Applications.

Donaldson, M. R., Cooke, S. J., Patterson, D. A., Macdonald, J. S. (2008). Cold shock and fish. Journal of Fish Biology, 73(7), 1491-1530.

Dulvy, N. K., Baum, J. K., Clarke, S., Compagno, L. J., Cortes, E., Domingo, A., ... Martínez, J. (2008). You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. Aquatic Conservation: Marine and Freshwater Ecosystems, 18(5), 459-482.

Dulvy, N. K., Fowler, S. L., Musick, J. A., Cavanagh, R. D., Kyne, P. M., Harrison, L. R., ... Pollock, C. M. (2014). Extinction risk and conservation of the world's sharks and rays. Elife, 3, e00590.

Edwards, J. R. (1988). The determinants and consequences of coping with stress. In: Causes, Coping Consequences of Stress at Work. Coopre CL and Payn R (eds). Chichester, John Wiley and Sons, pp.233-266.

Ellis, J. R., McCully Phillips, S. R., Poisson, F. (2017). A review of capture and post-release mortality of elasmobranchs. Journal of fish biology, 90(3), 653-722.

FAO (2011). International guidelines on bycatch management and reduction of discards. 73 pp.

Ferretti, F., Myers, R. A., Serena, F., Lotze, H. K. (2008). Loss of large predatory sharks from the Mediterranean Sea. Conservation Biology, 22(4), 952-964.

Figueiredo J. L. (1997). Manual de peixes marinhos do sudeste do Brasil. I. Introdução: Cações, raias e quimeras. São Paulo: Museu de Zoologia da Universidade de São Paulo.
Fitzsimons, J. T. (1979) The Physiology of Thirst and Sodium Appetite, pp. 1–121. Cambridge: Cambridge University Press.

Foden, W., Mace, G., Vie,´ J-C. et al. (2008). Species susceptibility to climate change impacts. In: The 2008 review of the IUCN Red List of Threatened Species. (eds) Vie,´ J-C., Hilton-Taylor, C., Stuart, S. N., IUCN, Gland.

Fowler, S.L.; Cavanagh, R.D.; Camhi, M.; Burgess, G.H.; Cailliet, G.M.; Fordham, S.V.; Simpfendorfer, C.A. Musick, J.A. (2005). Sharks, Rays and Chimaeras: The Status of the Chondrichthyan Fishes. International Union for Conservation of Nature and Natural Resources. pp. 106–109, 293–295.

French, S. S., DeNardo, D. F., Moore, M. C. (2007). Trade-Offs between the Reproductive and Immune Systems: Facultative Responses to Resources or Obligate Responses to Reproduction?. The American Naturalist, 170(1), 79-89.

Frick, L. H., Reina, R. D., Walker, T. I. (2010). Stress related physiological changes and post-release survival of Port Jackson sharks (*Heterodontu sportusjacksoni*) and gummy sharks (*Mustelus antarcticus*) following gill-net and longline capture in captivity. Journal of Experimental Marine Biology and Ecology, 385, 29–37.

Foo, J. T. W., Lam, T. J. (1993). Serum cortisol response to handling stress and the effect of cortisol implantation on testosterone level in the tilapia *Oreocrhomis mossambicus*, Aquaculture, 11(5), 145-158.

Fuentes, J., Eddy, F. B. (1997). Drinking in marine, euryhaline and freshwater teleost fish. In Ionic Regulation in Animals: A Tribute to Professor WTW Potts (pp. 135-149). Springer Berlin Heidelberg.

Fussel, H. M., Klein, R. J. T. (2006). Climate change vulnerability assessments: an evolution of conceptual thinking. Climatic Change, 75, 301–329.

Gallagher, A. J., Hammerschlag, N., Shiffman, D. S., Giery, S. T. (2014). Evolved for extinction: the cost and conservation implications of specialization in hammerhead sharks. BioScience, 64, 619-624.

Gallagher, A. J., Serafy, J. E., Cooke, S. J., Hammerschlag, N. (2014). Physiological stress response, reflex impairment, and survival of five sympatric shark species following experimental capture and release. Marine Ecology Progress Series, 496, 207-218.

Gingerich, A. J., Cooke, S. J., Hanson, K. C., Donaldson, M. R., Hasler, C. T., Suski, C. D., Arlinghaus, R. (2007). Evaluation of the interactive effects of air exposure duration and water temperature on the condition and survival of angled and released fish. Fisheries Research, 86(2), 169-178.

Gnaiger, E. (1983). Heat dissipation and energetic efficiency in animal anoxibiosis: economy contra power. Journal of Experimental Zoology (A), 228(3), 471-490.

Goldman, K. J. (1997). Regulation of body temperature in the white shark, Carcharodon carcharias. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology, 167(6), 423-429.

Goldstein, L. Forster, R. P. (1971). Osmoregulation and urea metabolism in the little skate, *Raja erinacea*. American Journal of Physiology, 220, 742–746.

Graham, F., Rynne, P., Estevanez, M., Luo, J., Ault, J. S., Hammerschlag, N. (2016). Use of marine protected areas and exclusive economic zones in the subtropical western North Atlantic Ocean by large highly mobile sharks. Diversity and Distributions, 1-13.

Guida, L., Walker, T. I., Reina, R. D. (2016). Temperature Insensitivity and Behavioural Reduction of the Physiological Stress Response to Longline Capture by the Gummy Shark, *Mustelus antarcticus*. PLoS ONE 11(2): e0148829.

Guida, L., Awruch, C., Walker, T. I., & Reina, R. D. (2017). Prenatal stress from trawl capture affects mothers and neonates: a case study using the southern fiddler ray (Trygonorrhina dumerilii). Scientific Reports, 7.

Gutierrez, J., Carrillo, M., Zanuy, S., Planas, J. (1984). Daily rhythms of insulin and glucose levels in the plasma of sea bass *Dicentrarchus labrax* after experimental feeding. General and Comparative Endocrinology, 55(3), 393-7.

Hammerschlag, N. (2006). Osmoregulation in elasmobranchs: a review for fish biologists, behaviourists and ecologists. Marine and Freshwater Behavior and Physiology, 39(3), 209-228.

Hammerschlag, N., Sulikowski, J. (2011). Killing for conservation: the need for alternatives to lethal sampling of apex predatory sharks. Endangered Species Research, 14(2), 135-140.
Hammerschlag, N., Gallagher, A. J., Lazarre, D. M. (2011). A review of shark satellite tagging studies. Journal of Experimental Marine Biology and Ecology, 398(1), 1-8.

Hall, M. (1996). On bycatches. Reviews in Fish Biology and Fisheries, 6, 319-352.

Hausfather, Z. (2004). India's shark trade: an analysis of Indian shark landings based on shark fin exports.

Harvell, C.D. (2002). Climate warming and disease risks for terrestrial and marine biota. Science, 296, 2158–2162 2

Haywood, G. P. (1973). Hypo-osmotic regulation coupled with reduced metabolic urea in the dogfish *Poroderma africanum*: an analysis of serum osmolarity, chloride, and urea. Marine Biology, 23(2), 121-127.

Hazin, F. H. V., Afonso, A. S. (2013). A green strategy for shark attack mitigation off Recife, Brazil. Animal Conservation ,17(4), 287–296.

Hazon, N. Henderson, I. W. (1984). Secretory dynamics of 1α- hydroxycorticosterone in the elasmobranch fish, *Scyliorhinus canicula*. Journal of Endocrinology, 103, 205–211.

Heard, M., Van Rijn, J. A., Reina, R. D. Huveneers, C. (2014). Impacts of crowding, trawl duration and air exposure on the physiology of stingarees (family: Urolophidae). Conservation Physiology, 2, 1–14.

Heberer, C., Aalbers, S. A., Bernal, D., Kohin, S., DiFiore, B., Sepulveda, C. A. (2010). Insights into catch-and-release survivorship and stress-induced blood biochemistry of common thresher sharks (*Alopias vulpinus*) captured in the southern California recreational fishery. Fisheries Research, 106, 495–500.

Heupel, M. R., & Simpfendorfer, C. A. (2002). Estimation of mortality of juvenile blacktip sharks, *Carcharhinus limbatus*, within a nursery area using telemetry data. Canadian Journal of Fisheries and Aquatic Sciences, 59(4), 624-632.

Heupel, M. R., & Hueter, R. E. (2002). Importance of prey density in relation to the movement patterns of juvenile blacktip sharks (*Carcharhinus limbatus*) within a coastal nursery area. Marine and Freshwater Research, 53(2), 543-550.

Heupel, M. R., Knip, D. M., Simpfendorfer, C. A., Dulvy, N. K. (2014). Sizing up the ecological role of sharks as predators. Marine Ecology Progress Series, 495, 291-298.

Hiddink, J. G., Hofstede, R. T. (2008). Climate induced increases in species richness of marine fishes. Global Change Biology 14, 453–460.

Hoegh-Guldberg, O., Mumby, P. J., Hooten, A. J., Steneck, R. S., Greenfield, P., Gomez, E., ... Knowlton, N. (2007). Coral reefs under rapid climate change and ocean acidification.

Hoffmayer, E.R., Parsons, G.R. (2001). The physiological response to capture and handling stress in the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*. Fish Physiology and Biochemistry, 25, 277–285.

Hoffmayer, E. R., Hendon, J. M., Parsons, G. R. (2012). Seasonal modulation in the secondary stress response of a carcharhinid shark, *Rhizoprionodon terraenovae*. Comparative Biochemistry and Physiology (A), 162(2), 81-87.

Hoffmayer, E. R., Hendon, J. M., Parsons, G. R., Driggers, W. B., Campbell, M. D. (2015). A comparison of single and multiple stressor protocols to assess acute stress in a coastal shark species, *Rhizoprionodon terraenovae*. Fish Physiology and Biochemistry, 41(5), 1253-60.

Hughes, T. P., Baird, A. H., Bellwood, D. R., Card, M., Connolly, S. R., Folke, C., ... Lough, J. M. (2003). Climate change, human impacts, and the resilience of coral reefs. Science, 301(5635), 929-933.

Hyatt, M. W., Anderson, P. A., O'Donnell, P. M. (2016). Behavioral release condition score of bull and bonnet head sharks as a coarse indicator of stress. Journal of Coastal Research, 32(6), 1464-1472.

Idler, D. R., Truscott, B. (1966). 1α-hydroxycorticosterone from cartilaginous fish: a new adrenal steroid in blood. Journal of the Fisheries Research Board of Canada, 23(4), 615-619.

IPCC Fifth Assessment Report - Impacts, Adaptation and Vulnerability. 2014. Available: https://www.ipcc.ch/publications_and_data/ar4/wg1/en/spmsspm-projections-of.html

Kajiura, S. M., Forni, J. B., Summers AP. (2003). Maneuvering in juvenile carcharhinid and sphyrnid sharks: the role of the hammerhead shark cephalofoil. Zoology, 106, 19-28.

Kajiura, S. M., & Tellman, S. L. (2016). Quantification of massive seasonal aggregations of blacktip sharks (*Carcharhinus limbatus*) in Southeast Florida. PloS one, 11(3), e0150911.

Kalmijn, A. J. (1971). The electric sense of sharks and rays. Journal of Experimental Biology, 71, 55 371-383.

Kirschner, L. B. (1993). The energetics of osmotic regulation in ureotelic and hypoosmotic fishes. Journal of Experimental Zoology, 267, 19–26.

Kleypas, J. A., Buddemeier, R. W., Archer, D., Gattuso, J. P., Langdon, C., Opdyke, B. N. (1999). Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. Science, 284(5411), 118-120.

Kleypas, J. A., Feely, R. A., Fabry, V. J., Langdon, C., Sabine, C. L., Robbins, L. L. (2005). Impacts of ocean acidification on coral reefs and other marine calcifiers: a guide for future research. In Report of a workshop held, 18, pp. 20.

Kirschner, L. B. (1993). The energetics of osmotic regulation in ureotelic and hypoosmotic fishes. Journal of Experimental Zoology, 267, 19–26.

Krustrup, P., González-Alonso, J., Quistorff, B., Bangsbo, J. (2001). Muscle heat production and anaerobic energy turnover during repeated intense dynamic exercise in humans. The Journal of Physiology, 536(3), 947-956.

Kuraoka K., Nakamura K. (2011). The use of nasal skin temperature measurements in studying emotion in macaque monkeys. Physiology and Behavior, 102, 347–355.

Lee, Z., Du, K., Arnone, R., Liew, S., Penta, B. (2005). Penetration of solar radiation in the upper ocean: A numerical model for oceanic and coastal waters. Journal of Geophysical Research: Oceans, 110(C9).

Lim, D. D., Motta, P., Mara, K., Martin, A. P. (2010). Phylogeny of hammerhead sharks (Family Sphyrnidae) inferred from mitochondrial and nuclear genes. Molecular Phylogenetics and Evolution, 55(2), 572-579.

Lin, J., Lee, Z., Ondrusek, M., Kahru, M. (2016). Attenuation coefficient of usable solar radiation of the global oceans. Journal of Geophysical Research: Oceans.

Lowe, C., Goodman-Lowe, G. (1996). Sun tanning in hammerhead sharks. Nature, 383, 677.

Malcolm, J. R., Liu, C., Neilson, R. P., Hansen, L., Hannah, L. E. E. (2006). Global warming and extinctions of endemic species from biodiversity hotspots. Conservation biology, 20(2), 538-548.

Mandelman, J. W., Farrington, M. A. (2007). The physiological status and mortality associated with otter-trawl capture, transport, and captivity of an exploited elasmobranch, *Squalus acanthias*. ICES Journal of Marine Science, 64, 122–130.

Mandelman, J. W., Skomal, G. B. (2009). Differential sensitivity to capture stress assessed by blood acid–base status in five carcharhinid sharks. Journal of Comparative Physiology (B), 179, 267–277.

Manire, C., Hueter, R., Hull, E., Spieler, R. (2001). Serological changes associated with gill-net capture and restraint in three species of sharks. Transactions of the American Fisheries Society, 130, 1038–1048.

Manire, C. A., Rasmussen, L. E. L., Maruska, K. P., Tricas, T. C. (2007). Sex, seasonal, and stress-related variations in elasmobranch corticosterone concentrations. Comparative Biochemistry and Physiology (A), 148(4), 926-935.

Marshall, W. S. (2003). Rapid regulation of NaCl secretion by estuarine teleost fish: coping strategies for short-duration freshwater exposures. Biochimica et Biophysica Acta: Reviews on Biomambranes, 1618(2), 95-105.

Marshall, W. S., Grosell, M. (2006). Ion transport, osmoregulation, and acid-base balance. In: The physiology of fishes. Evans DH, Claiborne JB, Currie S (eds). CRC Press, Boca Raton, USA, pp.177-230

Marshall, H., Field, L., Afiadata, A., Sepulveda, C., Skomal, G., Bernal, D. (2012). Hematological indicators of stress in longline-captured sharks. Comparative Biochemistry and Physiology (A), 162, 121–129.

Mauck, B., Bilgmann, K., Jones, D.D., Eysel, U. Dehnhardt, G. (2003). Thermal windows on the trunk of hauled-out seals: hot spots for thermoregulatory evaporation? Journal of Experimental Biology, 206, 1727–1738.

McCafferty, D. J., Currie, J., Sparling, C. E. (2007). The effect of instrument attachment on the surface temperature of juvenile grey seals (*Halichoerus grypus*) as measured by infrared thermography. Deep Sea Research Part II: Topical Studies in Oceanography, 54(3), 424-436.

McComb, D. M., Tricas, T. C., Kajiura, S. M. (2009). Enhanced visual fields in hammerhead sharks. Journal of Experimental Biology, 212, 4010-4018.

Meekan, M. G. (2016). Will the world's biggest fish get any bigger? Limits to the size of filter-feeding fishes over the last 160 million years. Science Proceedings, 34.

Mitchell, J. D., Collins, K. J., Miller, P. I., Suberg, L. A. (2014). Quantifying the impact of environmental variables upon catch per unit effort of the blue shark *Prionace glauca* in the western English Channel. Journal of fish biology, 85(3), 657-670.

Molina, J. M., Cooke, S. (2012). Trends in shark by catch research: current status and research needs. Reviews in Fish Biology and Fisheries, 22, 719-37.

Mommsen, T. P., Vijayan, M. M., Moon, T.W. (1999). Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology Fisheries, 9, 211–268.

Morgan, A., Burgess, G. H. (2007). At-vessel fishing mortality for six species of sharks caught in the Northwest Atlantic and Gulf of Mexico. Gulf and Caribbean Research, 19(2), 123-129.

Morgan, A., Carlson, J. K. (2010). Capture time, size and hooking mortality of bottom longline-caught sharks. Fisheries Research, 101(1), 32-37.

Moyes, C. D., Fragoso, N., Musyl, M. K., Brill, R. W. (2006). Predicting post release survival in large pelagic fish. Transactions of the American Fisheries Society, 135(5), 1389-1397.

Mueter, F. J., Litzow, M. A. (2008). Sea ice retreat alters the biogeography of the Bering Sea continental shelf. Ecological Applications 18(2), 309–320.

Musyl, M. K., Brill, R. W., Curran, D. S., Fragoso, N. M., McNaughton, L. M., Nielsen, A., ... Moyes, C. D. (2011). Post release survival, vertical and horizontal movements, and thermal habitats of five species of pelagic sharks in the central Pacific Ocean. Fishery Bulletin, 109(4), 341-368.

Nakaya, K. (1999). Hydrodynamic function of the head in the hammerhead sharks (Elasmobranchii: Sphyrnidae). Copeia, 330-336.

Nakayama, K., Goto, S., Kuraoka, K., Nakamura, K. (2005). Decrease in nasal temperature of rhesus monkeys (*Macaca mulatta*) in negative emotional state. Physiology and Behavior, 84, 783-790.

Naylor, G. J. P. (1992). The phylogenetic relationships among requiem and hammerhead sharks: inferring phylogeny when thousands of equally most parsimonious trees result. Cladistics, 8, 295-318.

Neiffer, D. L., & Stamper, M. A. (2009). Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. ILAR journal, 50(4), 343-360.

Nelson, P., Kajiura, S. M., Losey, G. (2003). Exposure to solar radiation may increase ocular UV-filtering in the juvenile scalloped hammerhead shark, *Sphyrna lewini*. Marine Biology, 142, 53-56.

Neves, K. R., Graciolli, F. G., Dos Reis, L. M., Pasqualucci, C. A., Moyses, R. M., Jorgetti, V. (2004). Adverse effects of hyperphosphatemia on myocardial hypertrophy, renal function, and bone in rats with renal failure. Kidney International, 66(6), 2237-2244.

Nowack, J., Dausmann, K.H., Mzilikazi, N., 2013. Non-shivering thermogenesis in the African lesser bush baby, Galago moholi. Journal of Experimental Biology, 216, 3811–3817.

Oide, H., Utida, S. (1968). Changes in intestinal absorption and renal excretion of water during adaptation to sea-water in the Japanese eel. Marine Biology, 1, 172–177.

Oliver, S., M. Braccini, M., Newman, S. J., Harvey, E. S. (2015). Global patterns in the bycatch of sharks and rays. Marine Policy, 54, 86-97.

Pabst, D. A., McLellan, W. A., Meagher, E. M., Westgate, A. J. (2002). Measuring Temperatures and Heat Flux from Dolphins in the Eastern Tropical Pacific: is Thermal Stress Associated with Chase and Capture in the ETP-tuna Purse Seine Fishery. LJ-02-34C, pp. 1–52. National Marine Fisheries Service NOAA, La Jolla, CA, USA.

Paladino, F. V., O'Connor, M. P., Spotila, J. R. (1990). Metabolism of leatherback turtles, gigantothermy, and thermoregulation of dinosaurs. Nature, 344(6269), 858-860.

Parmesan, C. (1996). Climate and species range. Nature, 382, 765–766.

Perrott, M. N., Grierson, C. E., Hazon, N., Balment, R. J. (1992). Drinking behaviour in sea water and fresh water teleosts, the role of the renin-angiotensin system. Fish physiology and biochemistry, 10(2), 161-168.

Perry, A.L., Low, P.J., Ellis, J.R., Reynolds, J.D. (2005). Climate change and distribution shifts in marine fishes. Science, 308, 1912–1915.

Pickering, A. D., Pottinger, T. G., Christie, P. (1982). Recovery of the brown trout, *Salmo trutta L*., from acute handling stress: a time-course study. Journal of Fish Biology, 20(2), 229-244.

Pikering, A. D., Pottinger, T. G. (1995). Biochemical effects of stress. In: Environmental and ecological biochemistry. Hochachka PW, Mommsen TP (eds.). Amsterdam: Elsevier, pp 349-379.

Poisson, F., Séret, B., Vernet, A. L., Goujon, M. Dagorn, L. (2014). Collaborative research: development of a manual on elasmobranch handling and release best practices in tropical tuna purse-seine fisheries. Marine Policy 44, 312–320.

Presscott, J. A., Collins, J. C. (1951). The Lag of Temperature Behind Solar Radiation. Quarterly Journal of the Royal Meteorological Society, 52, 121-126.

Prieto, J. I., Martínez-García, J. C., García, D. (2009). Correlation between global solar irradiation and air temperature in Asturias, Spain. Solar Energy, 83(7), 1076-1085.

Prosser, C.L., (1991). Environmental and Metabolic Animal Physiology. Wiley- Liss, New York.

Rasmussen, L. E. L., Crow, G. L. (1993). Serum corticosterone concentrations in immature captive whitetip reef sharks, *Triaenodon obesus*. Journal of Experimental Zoology, 267(3), 283-287.

Rasmussen, L. E. L., Gruber, S. H. (1993). Serum concentrations of reproductively-related circulating steroid hormones in the free-ranging lemon shark, *Negaprion brevirostris*. In: The reproduction and development of sharks, skates, rays and ratfishes, Demski, LS, Wourms, JP (eds). Springer Netherlands, pp167-174.

Randall DJ. (1982). The control of respiration and circulation in fish during exercise and hypoxia. Journal of Experimental Biology, 100, 275-88.

Rincon, G. (2007). A record of abortion in the school shark *Galeorhinus galeus* (Carcharhiniformes, Triakidae) captured on the continental shelf off southern Brazil. Pan-american Journal of Aquatic Science, 2, 53-54.

Roberto, J. V. B., de Souza, B. B. (2014). Utilização da termografia de infravermelho na medicina veterinária e na produção animal. Journal of Animal Behavior and Biometeorology, 2(3), 73-84.

Roemer, R. P., Gallagher, A. J., Hammerschlag, N. (2016). Shallow water tidal flat use and associated specialized foraging behavior the great hammerhead shark (*Sphyrna mokarran*). Marine and Freshwater Behavior and Physiology, 1-15.

Romero, M. L., Butler, L. K. (2007). Endocrinology of stress. Int J Comp Psychol, 20(2), 89-95.

Rosa, R., Seibel, B. A. (2008). Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator. Proceedings of the National Academy of Sciences, 10 (52), 20776-20780.

Rosa, R. S., Castro, A. L. F., Furtado, M., Monzini, J., Grubbs, R. D. (2006). *Ginglymostoma cirratum*. The IUCN Red List of Threatened Species 2006.

Sala, E. (2006). Top predators provide insurance against climate change. Trends in ecology evolution, 21(9), 479-480.

Schaller, G. B. (1968). Hunting behavior of the cheetah in the Serengeti National Park, Tanzania. African Journal of Ecology, 6 95-100.

Schluessel, V., Giles, J., Kyne, P. M. (2015). Notes on female reproductive biology and embryos of the brown guitarfish *Rhinobatos schlegelii* from the Penghu Islands, Taiwan. Ichthyology Research, 62, 342-357.

Serafy, J. E., Orbesen, E. S., Snodgrass, D. J., Beerkircher, L. R. Walter, J. F. (2012). Hooking survival of fishes captured by the United States Atlantic pelagic longline fishery: impact of the 2004 circle hook rule. Bulletin of Marine Science 88, 605–621.

Skomal, G. B. (2006). The Physiological Effects of Capture Stress on Post-Release Survivorship of Sharks, Tunas and Marlins. Boston, Massachusetts: Boston University, Ph.D. dissertation, 294pp.

Skomal, G. B. (2007). Evaluating the physiological and physical consequences of capture on post-release survivorship in large pelagic fishes. Fisheries Management Ecology, 14, 81–89.

Skomal, G. B., Lobel, P. S., Marshall, G. (2007). The use of animal-borne imaging to assess post release behavior as it relates to capture stress in grey reef sharks, *Carcharhinus amblyrhynchos*. Marine Technology Society Journal, 41, 44–48.

Skomal, G. B., Bernal, D. (2010). Physiological responses to stress in sharks. In: Sharks and Their Relatives II: Biodiversity, Adaptive Physiology, and Conservation. Carrier J, Musick J, Heithaus, M (eds). CRC Press, Boca Raton, USA, pp 459–490.

Skomal, G. B., Mandelman, J. W. (2012). The physiological response to anthropogenic stressors in marine elasmobranch fishes: a review with a focus on the secondary response. Comparative Biochemestry and Physiology (A), 162, 146–155.

Smith, M. F. (1992). Capture and transportation of elasmobranchs, with emphasis on the grey nurse shark (*Carcharias taurus*). Marine and Freshwater Research, 43, 325-343.

Smith, M. D., Thoney, W. D., R. Hueter, R. (2004). The Elasmobranch Husbandry Manual: Captive Care of Sharks, Rays and their Relatives. Columbus, OH: Special Publication of the Ohio Biological Survey.

Speers-Roesch, B., Treberg, J. R. (2010). The unusual energy metabolism of elasmobranch fishes. Comparative Biochemistry and Physiology (A), 155, 417-434.

Speers-Roesch, B., Richards, J. G., Brauner, C. J., Farrell, A. P., Hickey, A. J. R., Wang, Y. S., Renshaw, G. M. C. (2012). Hypoxia tolerance in elasmobranchs I. Critical oxygen tension as a measure of blood oxygen transport during hypoxia exposure. Journal of Experimental Biology, 215, 93-102.

Staurnes, M., Rainuzzo, J. R., Sigholt, T., Jorgensen, L. (1994). Acclimation of Atlantic cod (*Gadus morhua*) to cold water: stress response, osmoregulation, gill lipid composition and gill Na-K-ATPase activity. Comparative Biochemistry and Physiology (A), 109, 413–421.

Stevens, J., Bonfil, R., Dulvy, N. K., Walker, P. A. (2000). The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. ICES Journal of Marine Science, 57, 476-94.

Stevenson, R. D., Tuberty, S. R., Wingfield, J. C. (2005). Ecophysiology and conservation: the contribution of endocrinology and immunology–introduction to the symposium. Integrative and Comparative Biology, 45(1), 1-3.

Sundt, R. C., Meier, S., Jonsson, G., Sanni, S., Beyer, J. (2009). Development of a laboratory exposure system using marine fish to carry out realistic effect studies with produced water discharged from offshore oil production. Marine pollution bulletin, 58(9), 1382-1388.

Suski, C.D., Killen, S.S., Cooke, S.J., Kieffer, J.D., Philipp, D.P., Tufts, B.L., (2004). Physiological significance of the weigh-in during live-release angling tournaments for largemouth bass. Transactions of the American Fisheries Society, 133, 1291–1303.

Takei, Y. (2000). Comparative physiology of body fluid regulation in vertebrates with special reference to thirst regulation. Japanese Journal of Physiology, 50, 171–186.

Tattersall, G. J., Leite, C. A., Sanders, C. E., Cadena, V., Andrade, D. V., Abe, A. S., Milsom, W. K. (2016). Seasonal reproductive endothermy in tegu lizards. Science advances, 2(1), e1500951.

Terrien J., Perret M., Aujard F. (2011). Behavioral thermoregulation in mammals: a review. Frontiers in Bioscience, 16, 1428–1444

Tester, A. L. Olfaction, gustation, and the common chemical sense in sharks. (1983). Sharks and Survival, 8, 255-282.

Thomas, P. M., Pankhurst, N. W., Bremner, H. A. (1999). The effect of stress and exercise on post-mortem biochemistry of Atlantic salmon and rainbow trout. Journal of Fish Biology, 54(6), 1177-1196.

Thomas, C.D. et al. (2004). Extinction risk from climate change. Nature, 427, 145–148.

Thompson, L.J., Brown, M., Downs, C.T., 2015. The potential effects of climate-change associated temperature increases on the metabolic rate of a small Afrotropical bird. Journal of Experimental Biology, 218, 1504–1512.

Thums, M., Meekan, M., Stevens, J., Wilson, S., Polovina, J. (2012). Evidence for behavioural thermoregulation by the world's largest fish. Journal of The Royal Society Interface, rsif20120477.

Thuiller, W., Midgley, G. F., Hughes, G. O., Bomhard, B., Drew, G., Rutherford, M. C., Woodward, F. (2006). Endemic species and ecosystem sensitivity to climate change in Namibia. Global Change Biology, 12(5), 759-776.

Turner, T. A. (2001). Diagnostic thermography. Veterinary Clinics of North America-Equine Practice, 17(1), 95-114.

Treberg, J. R., Speers-Roesch, B. (2016). Does the physiology of chondrichthyan fishes constrain their distribution in the deep-sea? Journal of Experimental Biology, 219, 615-625.

Tricas, T. C., Kajiura, S. M., Summers, A. P. (2009). Response of the hammerhead shark olfactory epithelium to amino acid stimuli. Journal of Comparative Physiology (A), 195, 947-954.

Usamentiaga, R., Venegas, P., Guerediaga, J., Vega, L., Molleda, J., Bulnes, F. G. (2014). Infrared thermography for temperature measurement and non-destructive testing. Sensors, 14(7), 12305-12348.

Vitousek, M.N., Jenkins, B. R., Safran, R. J. (2014). Stress and success: Individual differences in the glucocorticoid stress response predict behavior and reproductive success under high predation risk. Hormones and Behavior, 66(5), 812-819.

Vooren, C.M., Lamónaca, A.F., Massa, A. Hozbor, N. (2006). *Zapteryx brevirostris*. The IUCN Red List of Threatened Species 2006: Downloaded on 18 November 2016.

Walther, G. et al. (2002). Ecological responses to recent climate change. Nature, 416, 389–395.

Walter, A. A. (1969). Relationship between Incoming Solar Radiation and Degree hours of Temperature. Agricultural Meteorology, 6, 435.

Waltman, B. (1966). Electrical properties and fine structure of the ampullary canals of Lorenzini. Acta Physiologica Scandinavica. Supplementum Journal, 264, 1.

Walsh, P. J., Kajimura, M., Mommsen, T. P., Wood, C. M. (2006) Metabolic organization and effects of feeding on enzyme activities of the dogfish shark (*Squalus acanthias*) rectal gland. Journal of Experimental Biology, 209, 2929–2938.

Wang, H., Wang, B., Normoyle, K. P., Jackson, K., Spitler, K., Sharrock, M. F., ... Du, R. (2014). Brain temperature and its fundamental properties: a review for clinical neuroscientists. Frontiers in neuroscience, 8, 307.

Weber, K., Sturmer, L., Hoover, E., Baker, S. (2007). The role of water temperature in hard clam aquaculture. UF/IAS Extension. Fisheries and Aquatic Science Department.

Wells, R. M. G., McIntyre, R. H., Morgan, A. K., Davie, P. S. (1986). Physiological stress responses in big gamefish after capture: observations on plasma chemistry and blood factors. Comparative Biochemistry and Physiology (A), 84, 565–571.

Whitlock, R. E., Hazen, E. L., Walli, A., Farwell, C., Bograd, S. J., Foley, D. G., Castleton, M., Block, B. A. (2015). Direct quantification of energy intake in an apex marine predator suggests physiology is a key driver of migrations. Science Advances 1(8):e1400270–e1400270.

Whitney, N. M., White, C. F., Gleiss, A. C., Schwieterman, G. D., Anderson, P., Hueter, R. E., Skomal, G. B. (2016). A novel method for determining post-release mortality, behavior, and recovery period using acceleration data loggers. Fisheries Research, 183, 210-221.

Whitney, N. M., Lear, K. O., Gaskins, L. C., Gleiss, A. C. (2016). The effects of temperature and swimming speed on the metabolic rate of the nurse shark (*Ginglymostoma cirratum*, Bonaterre). Journal of Experimental Marine Biology and Ecology, 477, 40-46.

Wikelski, M., Cooke, S. J. (2006). Conservation physiology. Trends in Ecology Evolution, 21(1), 38-46.

Williams, S. R., Stuarty, G. J. (1999). Mechanisms and consequences of action potential burst firing in rat neocortical pyramidal neurons. Journal of Physiology, 521, 467-482.

Wingfield, J. C., Romero, L. M. (2001). Adrenocortical responses to stress and their modulation in free-living vertebrates. Comprehensive Physiology.

Wingfield, J. C., Sapolsky, R. M. (2003). Reproduction and resistance to stress: when and how. Journal of neuroendocrinology, 15(8), 711-724.

Wright, A. J., Deak, T., Parsons, E. C. M. (2011). Size matters: management of stress responses and chronic stress in beaked whales and other marine mammals may require larger exclusion zones. Marine Pollution Bulletin, 63(1), 5-9.

Wright, P. A., Wood, C. M. (2015). Regulation of ions, acid-base, and nitrogenous wastes in Elasmobranchs. In: Physiology of Elasmobranch Fishes: Internal Processes Farrel AP, Brauner CJ (eds). Academic Press, London 279.

Wissing, H., Braun, H. A., Schafer, K. (1988). Dynamic response characteristics of the ampullae of Lorenzini to thermal and electrical stimuli. Progress in Brain Research, 74, 99-107.

Wittenburg, N., Baumeister, R. (1999). Thermal avoidance in Caenorhabditis elegans: an approach to the study of nociception. Proceedings of the National Academy of Sciences, 96(18), 10477-10482.

Wood, C. M., Turner, J. D., Graham, M. S. (1983). Why do fish die after severe exercise? Journal of Fish Biology, 22, 189-201.

Worm, B., Davis, B., Kettemer, L., Ward-Paige, C. A., Chapman, D., Heithaus, M. R., ... Gruber, S. H. (2013). Global catches, exploitation rates, and rebuilding options for sharks. Marine Policy, 40, 194-204.

Wosnick, N., Freire, C. A. (2013). Some euryhalinity may be more common than expected in marine elasmobranchs: The example of the South American skate *Zapteryx brevirostris* (Elasmobranchii, Rajiformes, Rhinobatidae). Comparative Biochemistry and Physiology (A), 166(1), 36-43.

Wosnick, N., Bornatowski, H., Ferraz, C., Afonso, A., Rangel, B. S., Hazin, F. H. V., Freire, C. A. (2016). Talking to the dead: using Post-mortem data in the assessment of stress in tiger sharks (*Galeocerdo cuvier*) (Péron and Lesueur, 1822). Fish Physiology and Biochemistry, 1-14.

Yopak, K. E., Lisney, T. J., Collin, S. P., Montgomery, J. C. (2007). Variation in brain organization and cerebellar foliation in chondrichthyans: sharks and holocephalans. Brain, Behavior and Evolution, 69, 280-300.

Yopak, K. E., Balls, G., Frank, L. (2009). Cortical surface structure analysis in sharks using magnetic resonance imaging (MRI). Proc ISMRM, 17, 2925.

Yopak, K. E. (2012). Neuroecology of cartilaginous fishes: the functional implications of brain scaling. Journal of Fish Biology, 80, 1968-2023.

Zagaglia, C. R., Damiano, C., Hazin, F. H. V., M. K. Broadhurst, M. K. (2011). Reproduction in *Mustelus canis* (Chondrichthyes: Triakidae) from an unexploited population off northern Brazil. Journal of Applied Ichthyology, 27, 25-29.